



# A TEXTBOOK OF HISTOLOGY

FUNCTIONAL SIGNIFICANCE OF CELLS AND  
INTERCELLULAR SUBSTANCES

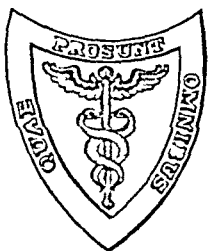
BY

E. V. COWDRY

PROFESSOR OF ANATOMY, THE SCHOOL OF MEDICINE, WASHINGTON UNIVERSITY, AND  
DIRECTOR OF RESEARCH, THE BARNARD FREE SKIN AND CANCER HOSPITAL,  
ST LOUIS, MO

*THIRD EDITION, THOROUGHLY REVISED*

Illustrated



LEA & FEBIGER  
PHILADELPHIA

COPYRIGHT  
IT' A & FFBIGER  
1944

---

Reprinted  
August 1946

PRINTED IN U S A

DEDICATED

TO THE MEMORY OF MY FATHER

N H. COWDRY

WHO, THOUGH BY PROFESSION A BANKER,

LED ME INTO THE WAYS OF SCIENCE





# PREFACE TO THE THIRD EDITION

---

IN this, and in previous editions, advice from friends has been very helpful. They now tell me that a simpler textbook of histology is needed, one in which many of the details are brushed aside so that the fundamentals are revealed in their true proportions, in fact, they suggest, a streamlined edition. The idea is not to save time by making it possible to hurry through the teaching of histology more quickly and with less resistance. It is, on the contrary, to permit a closer focussing of attention on the correlation of form and function by lightly passing over the minutiae which we as teachers must look up time and again because they are not part of our dynamic concept of the body in action. Consequently, and facing inevitable criticism, because there is so much to be learned and no two histologists view the body in exactly the same light, an effort is made in this edition more thoroughly to consider what in my opinion are the main correlations at the expense of the details mentioned.

But the method of presentation is unchanged. As before it centers about the blood vascular system as the principal integrator. Structure and function, being really indivisible, are described together in their natural fluid environments. When so connected a dynamic picture of the integrated whole gradually takes shape. This vision, however, will fade unless it is repeatedly called to mind and is ever made more comprehensive and accurate. The value of any course in the medical curriculum depends not only on its quality but also on the knowledge of it possessed by members of other departments and their action in building upon it.

I wish to thank my secretary, Miss Goessling, for her accuracy and patience, Mr J M Albrecht for skilled technical assistance, Mr W G Elle for his excellent photomicrographs and in particular my son, E V Cowdry, Jr, for helping me to understand the kind of assistance that medical students think they need.

ST. LOUIS, MO

E. V. C



# PREFACE TO THE FIRST EDITION

---

THE histology which we learned as students is very different from that we wish to present today. New discoveries come so thick and fast that there is danger of losing our perspective. But when we look backward, respect for the wisdom of the pioneers grows with every step forward. The time is passed when a broad, even presentation of the microscopic structure of the whole body is needed. Much that is important must be omitted in order to present the most vital subjects at all adequately. Justification for this is found by comparing standard works on physiology and histology. In the latter many pages are devoted to structures which the physiologist does not deem worthy even of mention. This elimination is significant. Concentration on a few subjects affords opportunity for the presentation of some of them in their proper setting, for emphasizing the value of experimentation and of what constitutes evidence.

What to omit and what to stress is the question. Obviously, the course in histology is not an isolated experience pursued for itself alone, but is an integral part of the entire medical curriculum. Most students have already had some training in biology in which cell division and the great truths of heredity and evolution are emphasized so that these need be considered only incidentally. The usual plan is to begin with a description of a typical animal cell, then to pass to the various tissues and finally to the several organs. This is illogical and time-consuming. A generalized kind of cell which exists alone and can be considered by itself is a figment of the imagination. The properties of each and every cell in the body are directly dependent upon its heredity, the fluid about it, and the other cells associated with it, and should only be investigated in this connection. The same applies to the so-called elementary tissues, epithelial, connective and so on. These, likewise, do not exist for themselves and by themselves, but function only insofar that they are integrated with the rest. Consequently an attempt is made to build up, as the discussion advances, a conception of cells and elementary tissues in the many environments in which they normally exist. Organs and systems are stressed in order to fulfill the principal aim of the volume, which is to relate structure and function in the whole body—itsself the real physiological unit.

A just criticism of most textbooks of histology is that they repeatedly illustrate the obvious. Figures consuming much space merely show at a glance what the actual preparations will lead the students to discover for themselves—a much more healthy experience. They are beyond the picture book stage. Good illustrations and clear-cut diagrams are, of course, invaluable, but they should present facts which the students find difficulty in unearthing, or which can only be discovered by experiments or techniques not practicable for them to make or apply.

Such figures with appropriate legends often speak for themselves. Space is also conserved by the use of tables which contrast the properties described. But an effort is made not to repeat unduly the same information in text, tables and illustrations. All the figures are of human tissues unless the contrary is stated.

As indicated in the list of contents, the central theme of the book is the blood vascular system—the great integrator. Wherever possible, organs in different stages of functional activity are compared so that a dynamic conception of the body will be given. The better textbooks of physiology include a good deal of histology and we do not hesitate, as amateurs, to try to interpret structure in terms of function. It is desirable to go even further and mention some structural modifications in pathologic states, for Nature is a good architect performing many significant adjustments in response to unusual demands. By learning how she overcomes special difficulties we can appreciate normal structure and function for better. The goal in view is that the students shall gradually visualize more and more accurately the wonderful reactivity of the minute structure of the human body in terms of biochemistry, physiology and pathology and finally in practice shall institute measures of assistance with due caution. All these experiences hang together. It is hoped that this book will prove useful beyond the scant two hundred hours or so devoted to the course in histology.

Controversial subjects have in no way been avoided, rather have they been sought out and aired. An effort has been made to present the evidence fairly and to acknowledge the labors of leading investigators. To mention specifically all contributors to histology would, however, be an impossible task. Neither is it feasible to discuss matters of priority. Sometimes the latest paper of an author is cited, relying upon him to mention his earlier contributions. References are given to the literature which will be most helpful to the student, especially those to papers with up-to-date bibliographies.

Help has been received from so many quarters that it is difficult to give adequate acknowledgment. Parts of the manuscript have been read by Drs. Edgar Allen, Walter C. Alvarez, R. R. Bensley, R. S. Cunningham, Harvey Cushing, A. W. Ham, F. B. Krumholz, I. C. Mann, C. C. Micklin, David Marine, A. T. Rasmussen, J. M. Russell and Eric Schour. The advice received has enabled me to avoid making many blunders but the responsibility for the book as it stands remains. For in rare instances I have relied on my own judgment. Original photomicrographs have been lent by many friends and metal cuts by several publishers as stated in the text.

My colleagues, Drs. D. F. Koevman, A. M. Lucas, M. S. Lucas, J. L. O'Leary and G. H. Seitz, have aided me from the very beginning. Mrs. I. A. Olch has rendered splendid service in the preparation of the manuscript and Miss Janice M. Richards has done artistic work. Finally Mr. W. D. Wilcox, representing the publishers, has given excellent assistance.

# CONTENTS

---

## ORIENTATION

Blood 12, Tissue fluid 13, Lymph 16

## BLOOD THE PRINCIPAL INTEGRATOR

### CHAPTER

#### I WHITE BLOOD CELLS

Identification unstained 17, Supravital staining 20, Blood smears 20, Differential leucocyte counts 21, Neutrophiles 23, Eosinophiles 27, Basophiles 29, Lymphocytes 30, Monocytes 31, Summary 38

#### II RED CELLS AND OTHER FORMED BODIES

Red cells 40, Platelets 45, Chylomicrons 47, Other particles 48, Summary 48

#### III BONE MARROW

General properties 49, Identification of cells 50, Cell lives 53, Numerical increase 54, Differential counts 55, Summary 55

## MECHANISM OF CIRCULATION

#### IV BLOOD VESSELS

Elastic arteries 57, Muscular arteries 60, Arterioles 63, Capillaries 66, Venules 68, Veins 69, Special adaptations 71, Summary 74

#### V HEART

Endocardium 77, Myocardium 77, Epicardium 78, Pericardium 78, Valves 79, Purkinje system 80, Maintenance 81, Summary 82

## DRAINAGE INTO BLOOD

#### VI LYMPHATIC SYSTEM

Lymphatic capillaries 84, Lymphatic vessels 84, Subepithelial lymphatic tissue 86, Lymph nodes 89, Evolution 94, Summary 94

#### VII SPECIAL LYMPHATIC ORGANS

Spleen 95, Microscopic landmarks 95, Splenic lobules 97, Lymphatic tissue 98, Red pulp 99, Reticulo-endothelial system 102, Thymus 103, Young and old 104, Function 107, Comparison of lymphatic organs 107, Summary 107

## CHEMICAL BROADCASTING VIA THE BLOOD

#### VIII ENDOCRINE SYSTEM

Thyroid 109, Parathyroids 115, Adrenals 121, Pituitary 127, Pineal body 135, Summary 136

## INTAKE OF MATERIAL AND REMOVAL OF WASTE

#### IX UPPER ALIMENTARY TRACT

Oral cavity 138, Teeth 139, Salivary glands 147, Tongue 150, Pharynx 153, Esophagus 153, Summary 153

#### X LOWER ALIMENTARY TRACT

Abdominal cavity 154, Stomach 157, Small intestine 162, Large intestine 171, Summary 175

## CHAPTER

## VI GLANDULAR APPENDICES

Pancreas 177, Liver 187 Summary 200

## OXYGEN INTAKE AND CARBON DIOXIDE ELIMINATION

## VII RESPIRATORY SYSTEM

Nasal passages 201 Paranasal sinuses 207 Pharynx 207 Larynx 208 Trachea 208 Bronchi 209 Bronchioles 209 Respiratory bronchioles 209 Alveolar ducts 210 Alveoli 210 Blood supply 214 Mechanical factors 215 Summary 216

## CIRCULATION OF COMPOSITION OF BLOOD

## VIII URINARY SYSTEM

Renal lobes 217 Lobules 218 Nephrons 219 Urinary passages 220, Summary 232

## RAPID INTEGRATION

## IX NERVOUS SYSTEM

Nerve cells 233 Dynamic polarization 242 Neuroglia 245 Membranes 245 Cerebrospinal fluid 246 Summary 248

## X PRINCIPAL SEXUAL ORGANS

Eye 250 Ear 250, Summary 253

## ORGANIZATION AND SUPPORT

## XI CONNECTIVE SYSTEM

Mesenchyme 255 Loose connective tissue 266 Fatty tissue 277, Cartilage 280 Bone 281 Articulations 290 Summary 302

## MOVEMENT

## XII MUSCULAR SYSTEM

Smooth muscle 303 Skeletal muscle 303 Cardiac muscle 312 Purkinje muscle 315 Summary 315

## DIFFERENTIATION OF RACE

## XIII MALE REPRODUCTIVE SYSTEM

Testicular architecture 316 Spermatogenesis 319 Sex hormone terminology 321 Hormone production 321 Tubuli recti rete testis and ductuli efferentes 327 Epididymis 327 Vas deferens 327 Vesiculae seminales 329 Prostate 331, Bulbo-urethral glands 334 Penis 334 Semen 336 Summary 338

## XIV FEMALE REPRODUCTIVE SYSTEM

Ovarian architecture 340 Oogenesis 341 Hormone production 348 Testes and ovaries compared 349 Fallopian tube 350 Uterus 353 Vagina 359 External genitalia 360 Mammary glands 361 Integration of activities 363 Nervous system 363 Regulation 364 Placenta 365 Summary 365

## UNIFICATION PROTECTION ADJUSTMENT

## XV SKIN

Epidermis 367 Dermis 367 Hairs 380 Sebaceous glands 380 Sweat glands 380 Nails 386 Summary 387

## XVI PERMEABILITY 390

# A TEXTBOOK OF HISTOLOGY

## ORIENTATION

IN histology the tendency is unavoidable to deal with the microscopically visible and consequently less fluid parts of the body. When the students excise living cells and hurriedly examine them in salt solutions, they often give but little thought to the complex body fluids in which the same cells normally transact their business. In the preparation of stained sections the tissues are almost completely deprived of water. A one-sided conception of vital processes may therefore develop, which it is desirable to counteract at the beginning. It would be difficult to suggest the rôle of fluids more graphically than in the words of the physiologist, Cannon (1930)

"A flowing stream brings to the simple organisms fixed on the rocks of the stream bed the food and oxygen needed for existence and carries away the waste. These single-celled creatures can live only in watery surroundings, if the stream dries they die or enter a dormant state. The same conditions prevail for the incalculable myriads of cells which constitute our bodies. We ordinarily think of ourselves as inhabitants of the air. In fact, however, every part of us that is alive is in contact with fluid. The surfaces of the body are either dead, as the horny layers of the skin, or are covered with moisture, as the eyes, the nose and the mouth. Within these surfaces are the vast multitudes of minute living elements or cells which comprise our muscles, glands, brain, nerves and other parts. Each cell has needs similar to those of the single cell in the flowing stream. But the body cells are shut away from any chances to obtain food, water and oxygen from the environment or to discharge the waste materials resulting from their activity. To provide these necessities moving streams of fluid have been developed to take from the moist surfaces of the body food, water and oxygen which they deliver to the cells in the remotest nooks of the organism, and from the cells they bring back to the moist surfaces the useless waste to be discharged."

As far back as 1859-1860 the French physiologist, Claude Bernard was teaching his medical students about the importance of the internal fluid environment. According to Cannon (1932) his opinion shifted as to just what this environment is. First, Bernard included only the blood plasma, then he added the lymph and finally included "the totality of the circulating fluids of the organism." He called it the *milieu interieur* and declared that "all vital mechanisms, however varied they may be, have but one object, that of preserving constant the conditions of life in the internal environment." J. B. S. Haldane believes that "No more pregnant sentence was ever framed by a physiologist" (Cannon, 1932).

This generalization has been the subject of many investigations, especially by Cannon and his associates. His book, "The Wisdom of the Body," should be familiar to every student of medicine. But histologists cannot agree with Cannon in his use of terms and in some other particulars. He states (p. 34) that "Lymph is produced by the filtering of a portion of the plasma through the capillary wall." The fluid thus originating is not in our view "*lymph*" but the intercellular or *tissue fluid* which directly bathes the cells. There is only one *lymph*, namely, the fluid within the lymphatics. Cannon also says that " . . . the lymph as a whole may enter a definite system of very thin walled tubes, the so-called lymphatics . . ." To enter as a whole would only be possible if the lymphatics were open



and this may be Cannon's idea because he does not mention the fact that in adults an endothelial barrier is interposed between the tissue fluid outside the lymphatics and the lymph within them.

We have therefore to consider not the totality of different fluids grouped under a single heading by Claude Bernard nor the blood and lymph of Cannon but three principal fluids namely blood tissue fluid and lymph.

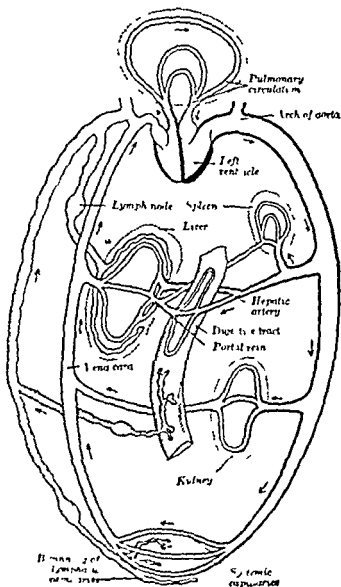


FIG. 1.—Diagram illustrating circulation of arterial blood (heavily outlined), venous blood (lightly outlined) and lymph (thinly outlined) after it has traversed a lymph node.

**Blood.**—The circulation of blood is indicated schematically in figure 1. Blood is received, charged with oxygen from the lungs into the right auricle which contracts and forces it on to the left ventricle. Contractions of the left ventricle drive it onward into the aorta and eventually in arterial streams to all vascularized tissues where it flows out in many capillaries. The capillaries in turn run together into veins which carry the blood to the right side of the heart whence it is pumped

into the lungs and the journey is repeated. Wherever it goes the blood is contained in a closed system of tubes lined everywhere by very thin cells, called endothelial. The analogy between the simple organisms in the flowing stream and tissue cells of the body breaks down because the latter are not in the blood stream; but are separated from it by this functionally very significant endothelial barrier. Only the blood cells are within the stream. The inner surfaces of the endothelial cells are washed by it.

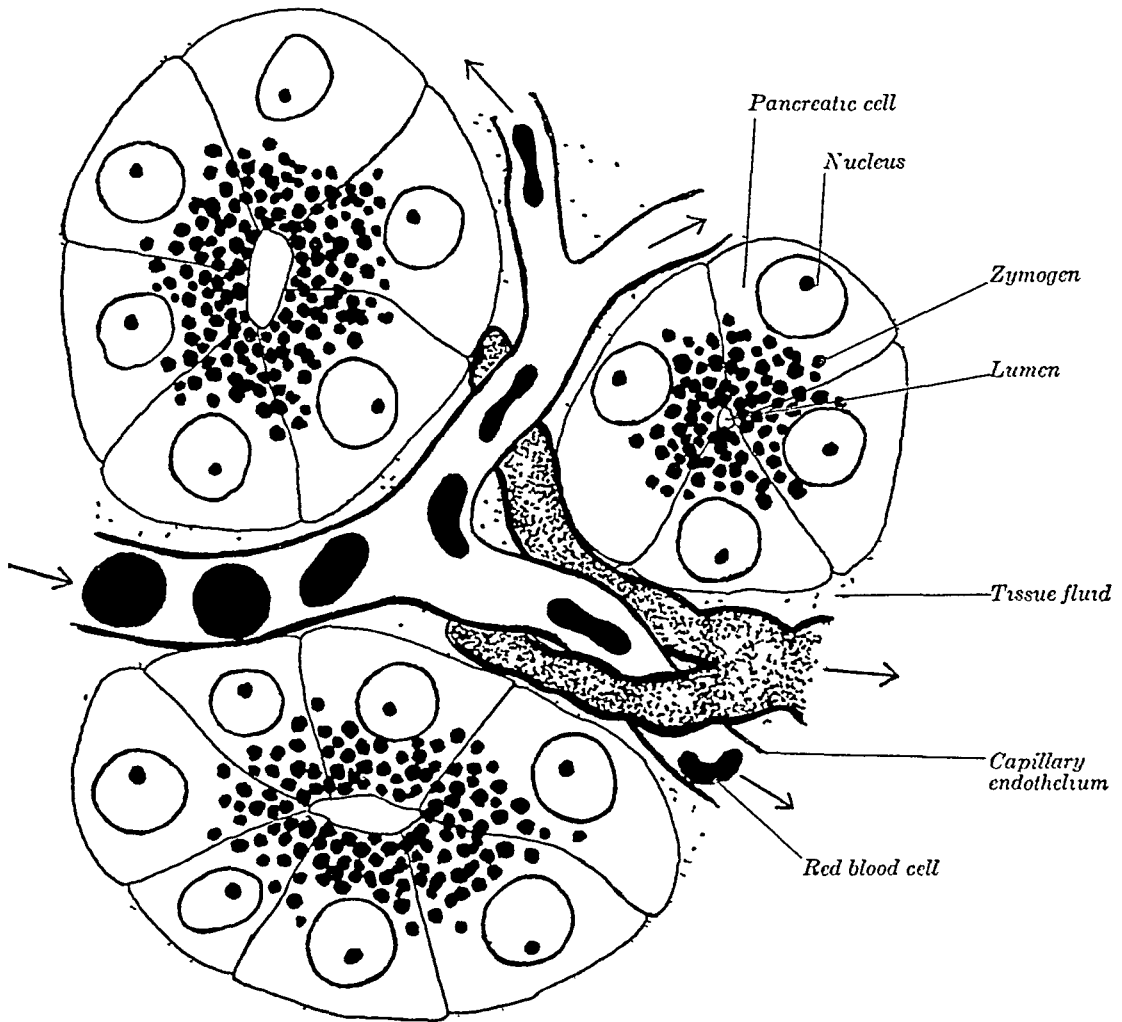


FIG. 2.—Diagram of interrelations of the three primary fluids. tissue fluid (fine stipple), blood (clear) and lymph (close stipple), as they are found in the pancreas. The arrows indicate the direction of flow of blood and lymph.

**Tissue Fluid.**—Extravascular cells throughout the body are inhabitants of tissue fluid. The position of this tissue fluid is indicated diagrammatically in figure 2 which shows, as viewed in section, three groups of pancreatic cells with their roughly spherical nuclei and small droplets of secretion antecedents (zymogen). The latter are grouped near the lumina into which the secretion is to be discharged. About the cells the tissue fluid is represented by fine stippling. It lies between them and the smooth endothelial wall of a capillary in which red blood cells are depicted in solid black. But the amount of tissue fluid in the pancreas is exaggerated in this diagram. In microscopic preparations of the normal pancreas there is so little of it that its location can hardly be seen. The groups of cells (acini)

appear to be closely pressed together—a little more so owing to shrinkage resulting from fixation than in the living state. Nevertheless materials passing from the blood stream must penetrate through the endothelium and enter the tissue fluid before they reach the cell membrane.

The tissue fluid is increased in edema as a consequence of increased permeability of the capillary wall so that more fluid is added to it from the blood stream. The space which it occupies is also rendered more evident when leucocytes and lymphocytes make their way into it from the blood. Such cells like those of the connective tissues are suspended in tissue fluid.

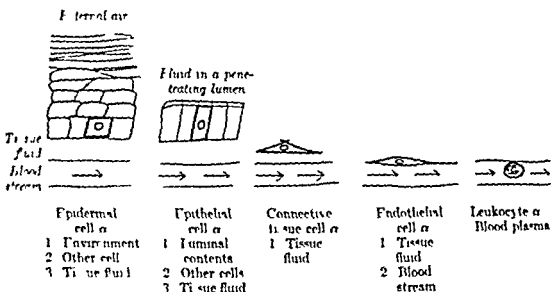


FIG. 3. Diagram showing different kinds of cellular adjustment.

A protozoan fixed to the rocks of the stream bed must hold tight to an inanimate surface and adapt itself to its fluid environment which is the water in the stream. The cells of our bodies are very much more sheltered and they vary with factors not operative in the same way in the external environment. Some of them are presented diagrammatically in figure 3.

A. An epidermal cell of the skin, the cell wall of which is heavily outlined, is protected from the external environment by layer upon layer of dead cells. It varies with (1) this environment to some extent, (2) other cells next to it and (3) the tissue fluid which exists between it and the blood stream and percolates slowly between it and its living neighbors.

B. An epithelial cell which lines a lumen connecting with the outside world is farther removed from the external environment. It is only exposed to those substances of external origin which run the gauntlet of various sphincters, digestive secretions and other protective mechanisms. It varies with (1) the material in the lumen, (2) other cells next to it and (3) the tissue fluid.

C. A connective tissue cell is more deeply placed. It is protected by epithelium from the external environment and the projections into the body of the above mentioned lumen. It varies with any cells or fibers with which it is in contact and with the tissue fluid which bathes it.

D. A vascular endothelial cell is still more sheltered, first by epithelium and then by the vessel wall. It varies with (1) the vessel wall and (2) the blood stream.

E A leucocyte in the circulating blood is most sheltered of all since it is protected by epithelium, tissue fluid and endothelium. It varies only with the blood plasma and other blood cells and endothelium with which it comes in contact.

It is evident that the vital adaptations of single-celled creatures in watery surroundings are fundamentally different from the cells which inhabit our bodies.

A survey of the body reveals great variability in the amount and character of the tissue fluid in different localities. It is always present in some measure between living cells though it may pass unnoticed. There is a fair amount in loose connective tissue (p 266). In cartilage and bone, tissue fluid becomes quite solid through accumulation of materials and fibers in it. In other places it is so conspicuous that it has been given special names—peritoneal, pleural and pericardial fluids, articular fluid in joints, etc. The cerebrospinal fluid, itself, can be considered as a special tissue fluid.

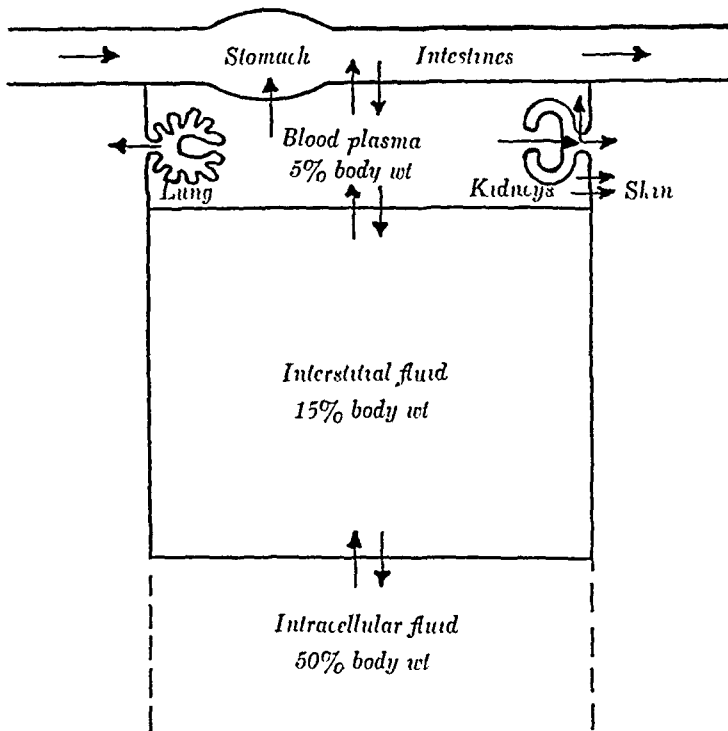


FIG 4 —Proportions of body fluids (after Gamble, 1937). The interstitial fluid includes both tissue fluid and lymph.

Local diversity in tissue fluid depends on several factors. (1) *The differential permeability of the blood vessels.* In some organs, like the liver, protein is allowed to enter the tissue fluid from the blood stream more readily than in others. (2) *The volume of the blood stream.* In highly vascularized organs the opportunity for diffusion through the endothelial barrier into the tissue fluid is greater than in organs possessing a poor blood supply or devoid altogether of capillaries (cartilage, cornea, epidermis). (3) *The special activities of the cells which inhabit it.* The cells derive their nourishment and chemical regulation immediately from the tissue fluid. The requirements of different sorts of cells are not the same so that the tissue fluid will differ. Similarly the waste eliminated by the cells into the tissue fluid will depend upon the functions they serve.

Upon the homeostatic stabilization in properties of the blood stream is, there-

fore, superposed an indispensable diversity in local tissue fluid environments without which division of labor and specialization of activity in the several organs and tissues could not be effected. This diversity in the fluid surroundings of living cells (other than those of the blood) in different tissues and organs is a gradual development during growth and differentiation made possible by the fact that the tissue fluids are comparatively stagnant and do not circulate like the blood. Whether they circulate sufficiently to come under Claude Bernard's heading of the totality of circulating fluids is a question.

**Lymph**—There is convincing evidence that in adults all lymphatic vessels are lined with endothelium. A physical barrier is consequently interposed between lymph (using the word correctly) and tissue fluid outside the lymphatics just as there is between the blood in the capillaries and the tissue fluid without only the endothelial barrier is not identical in both situations.

The lymphatic system is in a sense grafted upon the vascular system (see Fig. 1). It appears first in the vertebrates. Lymphatic capillaries begin blindly in the tissue fluid in the vicinity of blood capillaries. Such beginnings are heavily stippled in figure 2. The flow is always in one direction from the diverse tissue fluid environments of active cells toward the heart. Consequently the lymph from different regions is different just as the venous blood is different. The stream empties into the large veins at the base of the neck. As it passes haltingly in this direction it traverses various lymph nodes and lymphocytes are added. The blind beginnings of the lymphatics are often collapsed. The dilated state represented in figure 2, is unusual. Actually we must regard the lymphatics as a supplementary means of drainage of tissue fluid.

### SUMMARY

It is important to gain an idea of the course of fluid through the body and of the quantitative relations of the three fluids mentioned and of a fourth which is intracellular and completes the picture at least in rough outline. As indicated in figure 4 fluid enters the stomach via the esophagus and passes from the intestines into the blood stream where as blood plasma it amounts to about 5 per cent of body weight. Elimination is by the lungs into the stomach back into intestines through the kidneys and skin while a large amount enters the tissue fluid. This tissue fluid + lymph is vaguely called interstitial fluid and comprises approximately 15 per cent of the body by weight. There is an exchange between tissue fluid and intracellular fluid the latter being said to make up 50 per cent of body weight. Lymph and some tissue fluid pass back into the blood stream.

The average adult human being carries in his body about 100 lbs. of water. The amounts of extracellular and intracellular water are of great functional significance and can now be calculated with fair accuracy (Lowry and Hastings 1942). Certainly the system of supply to the cells and removal of waste from them is water borne and the body made up of fluids, cells and supporting structures formed by them is constantly changing in continuous adaptation to forces tending to upset its equilibrium. Some changes are rapid while others are slow, some are of large magnitude easily visible to the naked eye while others are ultramicroscopic. As the body ages it gradually adapts itself less well and wears out. It is for us in histology to obtain a dynamic concept of the minute structure of the body at work for until death work never ceases in the cellular population.

## CHAPTER I

### WHITE BLOOD CELLS

To begin the microscopic study of the body with leucocytes is logical from several points of view. Leucocytes are at once the most easily obtained and the most frequently examined of all cells. They can be directly studied alive in a small amount of the fluid in which they normally live and their behavior can be watched. Moreover it is a simple matter to identify all of the principal varieties without the use of any stains. Consequently the introduction to our cellular inhabitants is vital, direct and unobscured by artificial colors. There is another practical consideration. Oil immersion objectives and good illumination are essential so that a test of the students' microscopes is provided at the very beginning when their money can easily be refunded if necessary.

Each student should be provided with a small bottle containing 95 per cent alcohol. In the cork is inserted a needle of the Hagedorn variety with its cutting, lance-shaped end projecting into the alcohol. He should also have a covered dish partly filled with alcohol in which to keep slides and cover-glasses which have first been washed and then treated with cleaning fluid (Cowdry, *Microscopic Technique*, 1943, p. 53).

To avoid laboratory infections wash the hands in hot soap and water and the left index finger carefully with alcohol. Let the finger dry and puncture the skin of the lateral surface of the terminal phalanx (not the palmar surface because that is used in grasping). Touch the slide to the emerging blood and immediately cover. Practice is required in getting the right amount of blood.

**Identification Unstained.**—Leucocytes can be clearly distinguished from red blood cells by the absence of hemoglobin in them. Recognition of the straw-yellow color of hemoglobin, in individual red cells viewed with strong light, is therefore the first step. If this cannot be done, the individual is color blind and the sooner this fact is recognized and he learns to compensate by paying particular attention to form and to degree of brightness the better. Cells illustrated in the first row of figure 5, *A* are reds (more correctly referred to as erythrocytes) while the remainder are granular leucocytes. Of the latter, there are three types.

*Neutrophile leucocytes* (Fig. 5, *B*) are normally more numerous than any other type of white cell. Almost their entire cytoplasm is filled with fine granules, except the areas occupied by the nuclei, whose outlines are consequently sharply delineated. The nucleus is made up of a number of lobes connected by thin strands of nuclear substance, but the latter are difficult to see in fresh blood. This gives the impression that the cells are polynuclear, whereas they are actually polymorphonuclear. It will be noted that the neutrophiles are the first cells in the preparation to exhibit amoeboid movement. In thick mounts they are more rounded than in thin ones. In both they soon flatten out on the slide and crawl actively from place to place like slugs.

*Eosinophile leucocytes* (Fig. 5, *C*) are distinctly less numerous than the neutrophiles. This, coupled with the fact that their cytoplasmic granules are uniformly much larger and very highly refractile, renders identification a simple matter. The nucleus is likewise marked out by the absence of the granules. It is usually somewhat polymorphic but may be simple. The eosinophiles move about in a different way.

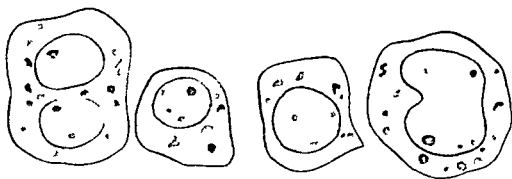
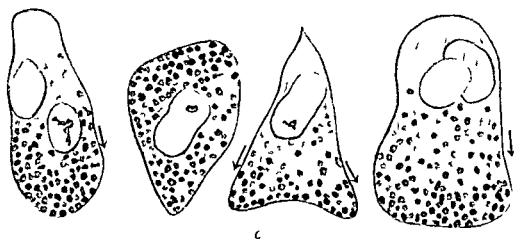
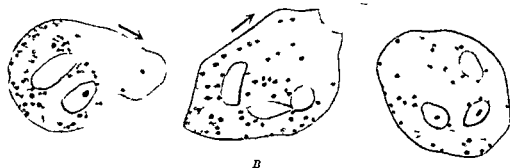
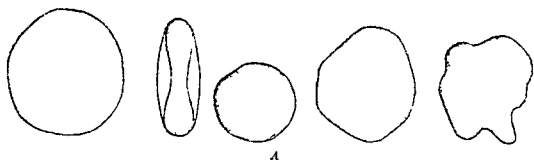


FIG. 3.—Camera lucida drawings of selected (A) erythrocytes (B) neutrophils (C) eosinophils and (D) basophils as seen in the living state unstained. The arrows indicate the direction they were moving at the time. The outlines are slightly accentuated in order to give a clear reproduction.  $\times 1600$

*Basophile leucocytes* (Fig 5, D) are the rarest of all white blood cells, occurring in the proportion of about 0.5 per cent. However, search for an hour or so of a number of mounts of blood generally brings one to light, although in the case of some individuals to find them is extraordinarily difficult. When seen they are unmistakable. Their granules are less densely packed and of variable size and shape. Under certain conditions the basophiles are also motile but less so than either of the other two.

*Lymphocytes* (Fig 6, A) are non-granular leucocytes in the sense that they do not possess specific granules comparable to those already mentioned. Numerically they stand between the neutrophils and the eosinophiles. The average lymphocyte is smaller than any of the granular leucocytes, though large forms may enter the blood occasionally. It contains a nucleus which may be spherical

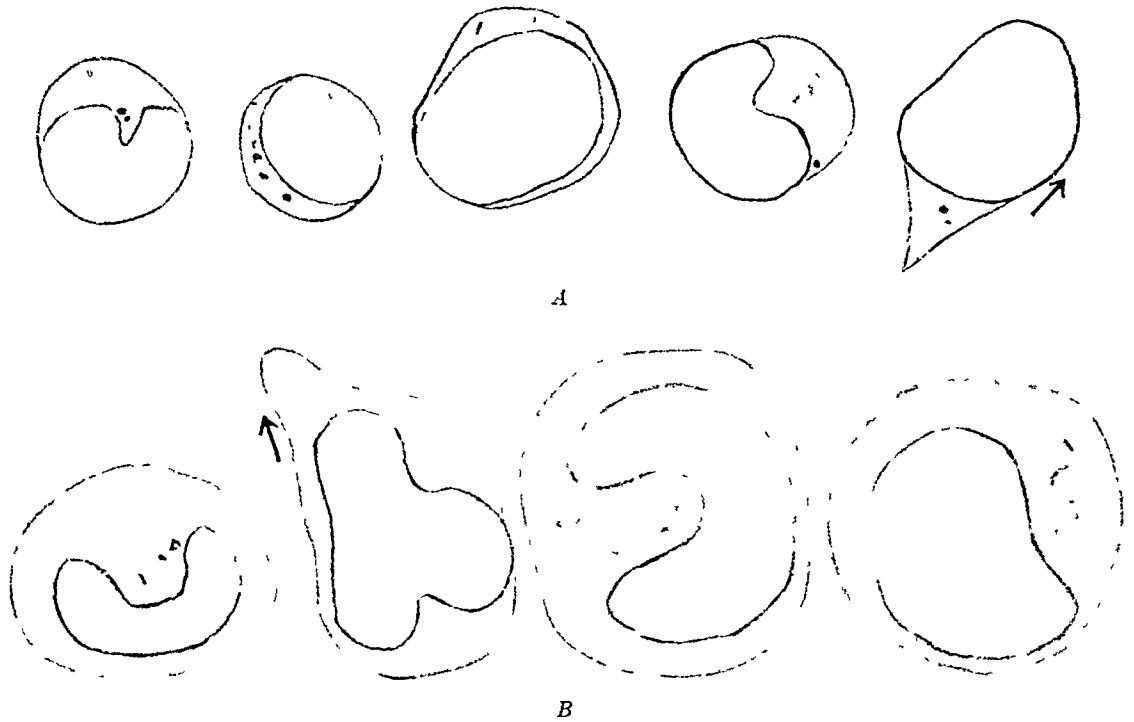


FIG 6—Camera lucida drawings of selected (A) lymphocytes and (B) monocytes as seen in the living state unstained  $\times 1600$

or slightly indented and occupies a proportionally very large part of the cytoplasm. The cytoplasm exhibits only a few granules, among which a little carbon is often noticeable in dwellers in smoky cities. This carbon identifies the lymphocytes originating in the pulmonary lymphatics for it is not found in other white cells. When first observed the lymphocytes differ from the three kinds of leucocytes by remaining stationary, but fifteen minutes or more after the blood has been drawn a few of them will be observed to move in a fashion quite different from the other cells described.

*Monocytes, or large mononuclear leucocytes* (Fig. 6, B), are not so readily identified in the living unstained state because some of them look like overgrown lymphocytes. The visible differences are more of degree than of kind. They are slightly more numerous than the eosinophiles and, normally, the lymphocytes are approximately twelve times more abundant than they are. The monocytes are the



largest cells generally encountered, while the lymphocytes are the smallest. Like the lymphocytes they possess a single large nucleus which is spherical or bean-shaped. It occupies relatively less of the cytoplasm than in lymphocytes. Granules occur in the cytoplasm and careful scrutiny shows that they are more numerous, smaller and rather more refractile than are the lymphocytic granules. The monocytes are motile but move in a different fashion from the lymphocytes.

The best way to demonstrate the characteristic structure and behavior of living unstained cells of these sorts is to show a moving picture film entitled 'Normal and Abnormal White Blood Cells in Tissue Cultures' by W. H. and M. R. Lewis in cooperation with A. R. Rich and M. M. Wintrobe, Carnegie Institution of Washington. This can be rented from the Wistar Institute of Anatomy at Philadelphia.

**Supravital Staining**—By the addition of dilute solutions of dyes to fresh blood other structural details can be demonstrated. Janus green is the most useful of these dyes because it stains mitochondria.

The technique is simple. A small drop of 1 to 10,000 solution of Janus green B (which must be diethyl afrazanazodimethylamin chloride) in physiological saline is placed on a slide. A very little blood is added and immediately covered with a cover glass. No attempt should be made to mix the blood with the stain. This is done by the pressure of the cover glass alone. Success will depend chiefly upon practice in securing such small amounts of both that the two are pressed into a very thin layer occupying all the space between the cover and the slide. Optimum coloration is obtained in five or ten minutes, first, of the mitochondria in the lymphocytes and, later, of those in the neutrophils. Another method is to allow some of the dye in alcohol or water to dry on the slides and then add a little blood and cover. The Janus green then goes into solution in the blood plasma and stains the mitochondria in much the same way. The addition of physiological saline to the blood cells is thus avoided. The mitochondria are colored light bluish green and are at first the only elements in the preparation stained at all. They occur in the form of tiny granules and rods which look something like bacteria (Technique, p. 123).

Neutrophils can be colored in the same way to color the so-called neutral red granule. Some hematologists use the two dyes together (for details see Histological Technique, p. 34).

**Blood Smears**—Preliminary to further work is the making and staining of blood smears which can be examined at leisure.

These should be made on slides rather than on cover glasses for several reasons. A larger film of blood is thereby provided for examination. Smears on slides are easier to make and to handle. They can be studied without covering them whereas a smear on a cover glass cannot be moved about on the stage of the microscope unless it is mounted smear side down on a slide. The colors are often more permanent in smears on slides which are not covered with cover glasses. A good way is to spread a thin film of immersion oil over them. This dries much more quickly than balsam or any other medium under a cover glass.

Slides of good quality with ground edges and scrupulously clean are necessary. A finger tip or ear lobule is first cleaned with 95 per cent alcohol. As soon as the surface has dried a small puncture is made with a previously sterilized needle. Special needles with lance-shaped cutting ends are better than ordinary pointed ones. A small droplet of blood should appear on slight pressure. The first is wiped away with sterile gauze and the second and following ones are used. Unless the blood is very strongly pressed out the differential count of white cells will not be affected. Some advise holding the fingers in hot water beforehand to produce a temporary hyperemia in them but this is seldom advisable. A droplet of size sufficient to produce a smear of the desired thickness (determined by trials) should be touched to the surface of a slide conveniently placed on a table about 3 cm. from one end. Immediately the end of a second slide, with its edge squarely across the first slide is brought in contact with the blood on the remote side of the drop from the nearest end of the first slide. The blood spreads quickly along this edge toward the sides of the slide on the table.

which is steadied with the left hand. The end edge of the second slide is slowly but steadily pushed the length of the first slide and the blood is drawn out in a thin layer after it. The angle of inclination of the second to the first slide determines the thickness of the smear. It is well to make the first smear at an angle of about 45 degrees, increase it for a thicker smear and decrease it for a thinner one. In the making of smears it is important to have plenty of elbow room. To make good smears is a fine art and a credit to the individual.

Blood smears retain their staining properties for a few days but they should be colored without undue delay. It is both wasteful and undesirable to cover the whole slide with stain. Part of the slide will have to be used for record written with a diamond pencil. Therefore draw two lines across the slide near each end with a wax pencil or a piece of paraffin

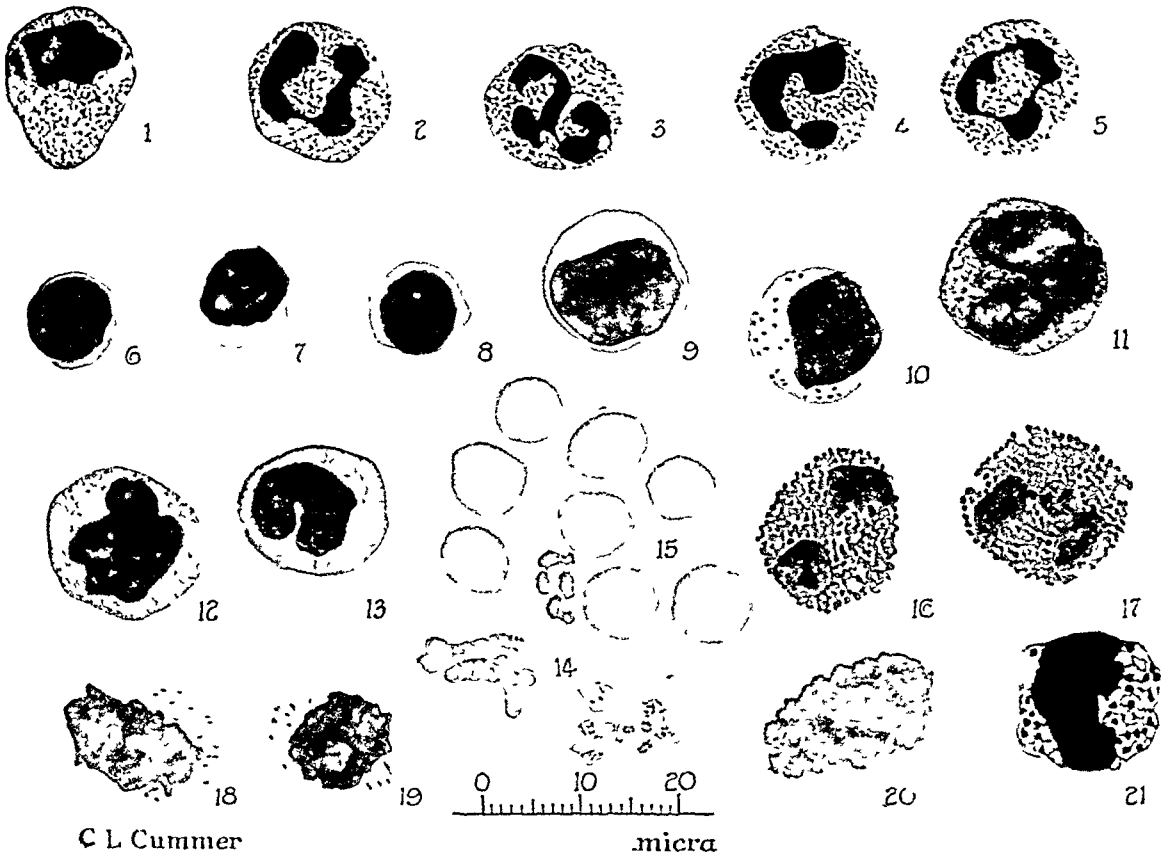


FIG 7—Types of cells found in normal blood. Colored by Wright's stain. All cells drawn with the same magnification and outlined with the camera lucida for the purpose of comparing sizes.  $\times 1150$ . Nos 1 to 5, inclusive, neutrophils; 6, 7, 8, lymphocytes; 9 to 13, monocytes; 14, platelets; 15, a group of red blood cells; 16, 17, eosinophils; 18, 19, 20 "basket cells," degenerated leucocytes; 21, basophilic leucocyte.

The stain added with a dropper will cover only the intervening part. Wright's blood stain (Commission Certified) is recommended. Dry the smear in air. Cover the area between the wax lines with stain measured by drops from a medicine dropper. After 1 min. add same volume aq. dest., shifting the slide a little from side to side so that it mixes fairly well. A green metallic looking seum forms on the surface. Leave 2 or 3 min. Too long staining produces a precipitate. It may be necessary to use for dilution instead of aq. dest. the McJunkin-Haden buffer. Wash in tap water 30 sec. or more until thin parts of smear become pink or yellow. Dry by blotting with smooth filter paper and examine directly without mounting in balsam and adding a cover-glass. (Technique, p. 33)

**Differential Leucocyte Counts.**—To gain familiarity with the leucocytes in stained smears, it is desirable to make *differential counts* of the kinds of leucocytes

already briefly characterized. The information gained is of interest to the owner of the blood and provides a basis for a more detailed discussion of each type of cell.

A student possessing a microscope equipped with a mechanical stage has a distinct advantage because he can be sure to only count the same area once. Select with low power an evenly spread and well stained smear. With the oil immersion passing from left to right and back again, classify the first 200 leucocytes encountered. As in the fresh preparations the neutrophils are identifiable by their tiny granules by their polymorphic nuclei which show much more clearly in the stained condition. The eosinophiles have distinctly larger granules colored reddish and their nuclei are likely to be a little less polymorphic and less deeply stained than those of the neutrophils. Normally there are about 20 neutrophils to 1 eosinophile. Basophiles are much more difficult to locate. This is due to their scarcity. But when one is found it is immediately recognized by its basophilic granules which are often few in number and of variable size. Since time is precious some students do not get a good view of basophiles until they examine bone marrow. By convention the small lymphocytes are arbitrarily considered to grade up to a red blood cell in diameter, the intermediate ones to be larger in diameter than one and less in diameter than three while the large ones are greater than three. Small lymphocytes are common in the blood, intermediates are scarce and large lymphocytes are not normally found therein. To distinguish between intermediate lymphocytes and monocytes is difficult and sometimes impossible. The latter usually have a more indented nucleus a less basophilic cytoplasm more conspicuous fine granules staining with the Azure component of the mixture (termed Azurophile) and when stained supravitally with neutral red and janus green exhibit a kind of rosette of "neutral red granules" in the nuclear concavity.

Such a differential count usually reveals approximately 68 per cent neutrophils 3 per cent eosinophiles 0-0.5 per cent basophiles 20-22 per cent lymphocytes and 5-7 per cent monocytes. The greatest variation is normally found in the lymphocytes which cannot be altogether explained by failure to properly distinguish between them and monocytes. A minority of medical students each year report 40-50 and even higher percentages. When the cells look atypical or the counts remain high when repeated at weekly intervals advice of Student Health Service should be obtained. Harris (1938) has shown that emotion may result in lymphocytosis.

Student	Before experiment	After stress	Practical histology	Written histology	Written anatomy	Oral anatomy
22	-----	-----	-----	-----	-----	51.8
21	-----	-----	-----	-----	51.2	-----
13	-----	-----	-----	46.07	-----	-----
12	-----	-----	40.8	-----	-----	-----
30	-----	35.9	-----	-----	-----	-----
31	24.4	-----	-----	-----	-----	-----
Total leucocytes	7389.4	7851.4	7458.3	835.0	7573	7300

Before the experiment started the average percentage in 31 students was 24.4. After the mild worry of a thesis it rose to 35.9. With examinations considered to be of increasing severity the average percentage climbed to 51.8 lymphocyte count. The lymphocytosis disappeared in from one-half to twenty-four hours following release from emotion.

Note that the total number of leucocytes showed no marked change during the experiment. When employed to designate such total counts (usually made in a later course in clinical microscopy) all white cells are included as leucocytes. However it is common practice also to speak of leucocytes in contrast to lymphocytes which are also white cells. One should always bear in mind the variations both quantitative and qualitative in normal leucocytes (Sturgis and Bethell 1917).

**Neutrophiles.**—In addition to their polymorphic deeply staining nuclei and tiny neutrophilic cytoplasmic granules, the neutrophilic leucocytes show no particular, easily seen, distinctive properties / As one would expect the general structural components, common to most cells, are also present, just as all humans have lungs, kidneys, arms, legs, etc Of these mitochondria are perhaps of most universal distribution so that their presence can be taken for granted and need not enter into subsequent descriptions except in special cases Their shape is indicated by the name (*G mitos*, thread and *chondros*, grain) They can be easily demonstrated by supravital staining with janus green and have been mistaken for bacteria Centrioles are rarely seen When present they occur in the concavity of the nucleus In the region of the centriole an accumulation of material, termed the Golgi apparatus, after its discoverer in nerve cells, can occasionally be demonstrated by special methods Its rôle in cellular activity remains to be determined

But only the grosser details of structure are revealed microscopically They afford but a poor and inadequate background on which to consider function which interests us most Because the physiologic activities of all cells are co-extensive with their individual lives, it is proper here to outline the events and opportunities in the life of a neutrophile He is the Ulysses among cells—a great wanderer

(1) *In the Bone Marrow*—Individual neutrophiles begin life as the daughter cells produced by the last division of neutrophilic myelocytes of which more later (p 51) Unfortunately this last division is not identifiable, neither is it possible to follow the whole career of a given neutrophile It is clear, however, that a neutrophile does not attain to quite the size of the parent myelocyte It is also evident that, before leaving the bone marrow and entering the blood stream, its nucleus becomes slightly more polymorphic At the same time it is fair to assume that motility and phagocytic power increase

While all neutrophilic leucocytes are ready to devote their lives to the welfare of the cellular community, not all of them are drafted for this purpose Their existence extends from mitotic birth through youth, maturity and senescence to death For this reason they are listed as *postmitotics* in contrast with their myelocytic parents which are *intermitotics*. The individual lives of the latter are fundamentally different Though they begin, likewise, with the mitosis that gives them birth, their existence as individuals extends between this mitosis and the next in which they divide into 2 daughter cells They do not attain full functional specialization neither do they become senescent and die but individuality ceases. This classification of cells according to their mode of life is given in detail elsewhere (Cowdry, 1942)

(2) *In the Blood Stream*—As neutrophiles are carried along they are capable of phagocytosing certain bacteria (if present) and some particulate materials with which they may chance to come in contact But the great majority seldom enjoy the opportunity of ingesting bacteria because blood infections are rare. More frequently a few are called into the tissue fluids to combat local infections

Exactly how long, on the average, neutrophiles remain in the circulation has not been discovered. Roberts and Kracke (1930) have observed a complete disappearance of neutrophiles from the circulation within four days after the decrease began in a patient suffering a recurrence of the condition of agranulocytosis If, during these four days, no young neutrophiles entered the blood from the bone marrow and those that were present disappeared at the same rate as in a normal person, their average time of sojourn in the blood stream could be placed at somewhat less

than four days Weiskotten (1930) has reported the disappearance of rabbit leucocytes which correspond to human neutrophils, from the circulation three to four days after the production of new cells was prevented by injury of the marrow with benzol The similarity in the time of disappearance lends support to both observations

Neutrophils in the circulation have been listed in five classes of increasing age by Arneth (1904) depending upon whether they possess 1, 2, 3, 4 or 5 (or more) nuclear lobes (Fig 8) In Schilling (1929) counts three types of neutrophils (juveniles stab nuclears and segment nuclears) are recognized, characterized by increasing nuclear polymorphism and by certain other features, in the transition from youth to maturity Valuable data on the maturity of neutrophils in the circulation are to be obtained from such counts But Bunting (1932) reminds us

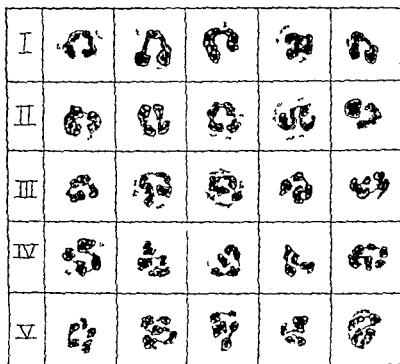


FIG 8 -Classification of leucocytes in five Arneth groups (Redrawn from Cooke, J Roy Mic Soc)

that leucocytes with basophilic protoplasm loosely woven nuclei and even with basophilic granules among the neutrophilic granules and thus obviously young cells may show as many lobes to the nucleus as cells evidently senile He also calls attention to the excessive lobation (8 or 9) in certain pathological states When neutrophils are examined at intervals in blood isolated in veins between two ligatures development from one class of Arneth to another is not observed, but when blood is thus isolated in which the number of young forms is first increased by stimulation of the bone marrow development from class I to class II can be demonstrated (Clumerko and Ponder 1934) According to Crossman and Charipper (1938) increased functional activity brings about an increased lobation in a shorter time than that required to produce a similar lobation with time alone as a factor More recently Richter (1942) removed blood from the axillary vein and examined

the neutrophiles after incubation for various lengths of time. In smears made at the times indicated (Fig 9) he found neutrophiles in decreasing stages of nuclear polymorphism.

Schilling (1908) was the first to notice non-motile neutrophiles in blood examined in the dark field. Sabin later observed similar cells in which she (Sabin *et al*, 1924) failed to find mitochondria with the help of janus green. Decrease in motility and in mitochondrial content are both to be expected as neutrophiles age but how general is the death of these cells in the blood stream remains to be determined. Smith and McDowell (1929) have been unable to confirm the idea that large numbers of them die and think that the dead, non-motile cells are often technical artefacts. According to Maximow and Bloom (1937), "The presence of degenerating leucocytes in the circulating blood, although often described, has never been confirmed experimentally."

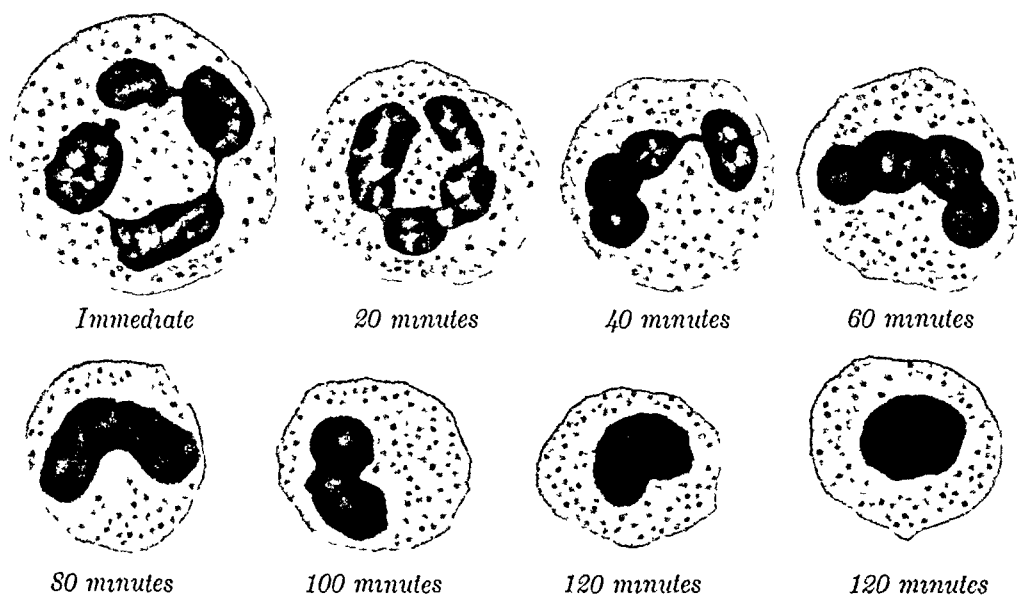


FIG 9 —Structure of neutrophiles when fixed and stained at various intervals after removal from axillary vein and incubation at body temperature  $\times 2220$  (From Richter, Courtesy of J. Morph.)

If three to four days is accepted as the duration of their residence in the blood stream,  $\frac{1}{3}$  to  $\frac{1}{4}$  of the total number present must leave the blood in some way each day. Many are filtered out by the spleen and the liver to be destroyed by reticulo-endothelial cells (p 102), but, in the absence of counts of non-motile leucocytes in blood entering and leaving these filters, two possibilities remain: either that these cells are removed with extraordinary speed from the circulation or that only a very small, almost negligible, proportion of neutrophiles age and die in this way because as seniles they are so seldom seen in the blood. The neutrophiles, which remove themselves from the circulation by entering tissue fluids, do not figure in this estimate because it is concerned only with the frequency of neutrophiles which lose their lives and cannot emigrate.

(3) *In Tissue Fluids* —Leucocytes may happen to be washed through vessels the endothelial linings of which have, for some reason, become sticky so that they adhere to the vessel walls (see p 69). Correlated with this stickiness, but sometimes independent of it, the blood stream may be slowed or temporarily stopped.

It would be a mistake to assume that either of these conditions is necessarily abnormal though both can result from injury. However they favor the passage of neutrophils through the vessel walls and their entrance into the surrounding tissue fluids. In pathological states this migration of neutrophils can occur in any part of the body in response to a chemical message (chemotaxis) so that the environments entered can be of wide variety especially since some of the environments probably change with ageing of the body. That some neutrophils normally enter certain tissue fluids is assured as will appear later.

The only detailed account of their age changes in tissue fluid we owe to the Clarks (1936). These investigators studied the finely granular leucocytes of rabbits which correspond to human neutrophils in special chambers inserted into the ears. After passing through the vessel walls the leucocytes move about in the tissue fluid for a day or two phagocytosing any materials that appeal to them, come to rest, round up, lose their nuclear polymorphism and before they die assume an appearance reminiscent of non granular leucocytes (lymphocytes). Richter's observations already noted are confirmatory.



FIG. 10—Physiological leucocytosis occurring during menstruation. Drawing of a smear from the third stage fluid showing the arrival of myriads of leucocytes into the vaginal fluid; the epithelial cells lie scattered among them and are being dissolved and invaded by the leucocytes. (Redrawn and modified from Stockard.) (Cowdry's Special Cytology, Paul B. Hoeber, Inc.)

(4) *In Lumina Connecting With the Outside World*—Though many neutrophils age and die in the tissue fluids, some travel further afield. Isaacs and Danielian (1927) have reported that neutrophils pass into the mouth in saliva and have considered it likely that they also escape through the mucous membrane into other parts of the alimentary tract. Perhaps in this anatomically extracorporeal location some neutrophils are still able to phagocytose bacteria. The authors concluded that this method of elimination is a regulatory mechanism for maintenance of the proper number in the blood. Bunting (1938) found neutrophils in the salivary ducts but did not commit himself on the conclusion reached by Isaacs and Danielian. The literature contains many accounts of similar escape by lymphocytes and eosinophiles.

Stockard's (1932) description of the migration of swarms of leucocytes of the same type (but not neutrophils in the mammals studied) into the vaginal fluid during the third stage of oestrus is particularly significant because it is a normal process, not at all in response to bacterial invasion (Fig. 10). According to him a special purpose of the leucocyte seems to be to destroy the excess of spermatozoa

remaining in the uterus      A leucocyte comes in contact with a spermatozoan which with its tail is longer than the leucocyte. The leucocyte by stretching and contracting finally takes into itself the entire spermatozoan, the tail being wound in a circular fashion within the cell body. Neither this genital, nor the alimentary escape, is possible until the neutrophils have first left the blood vessels and have entered the respective subepithelial tissue fluids.

Several facts emerge from this glimpse of the life history of neutrophils. First it is interesting that, though these cells may be living in many different environments, they usually present the same appearance and presumably are ready to function in the same way. Second, there is a large element of chance in their call to service. In the circulation they phagocytize certain bacteria with which they happen to come in contact—a rare experience. (Neutrophils are rich in enzymes, Barnes, 1940.) When present in the blood flowing through a portion of a vessel, the lining of which has for some reason become sticky, they may adhere to the sticky surface (Fig. 44). If, when in this position, they receive a chemotactic message from outside the vessels, they may respond and move through the wall into the surrounding tissue fluid (on the mechanism of chemotaxis, see McCutcheon, 1942). Or they may chance to be swept out into the tissue fluid with other elements of the blood if the wall of a vessel is cut or broken. Once in the tissue fluid their behavior is conditioned by the fluid, or particulate matter in the fluid, which touches them. Depending on circumstances, they may or may not become phagocytic. Perhaps by dying in healing wounds some liberate food substances for fibroblasts, which Carrel (1924) calls trephones, and thus make themselves useful. Conditioned by location of the tissue fluid they may, or may not, find themselves near lumina communicating with the outside world. If they do, and they happen to be among those beckoned onward, in some way difficult to understand, they may escape into alimentary and other fluids.

On the whole it is not surprising that neutrophils are provided in numerical excess of the ordinary functional demands for them—that there is a large factor of safety. In practice the number of neutrophils in a sample of blood is a valuable indicator of the condition of affairs in the body. If the total leucocyte count, made up as it normally is chiefly of neutrophils, increases greatly one looks for other evidence of an acute infection. If the Schilling count shows that the number of young neutrophils, with incompletely segmented nuclei, is greater than normal it is said to reveal a shift to the left which is a bad sign. This indicates that the need for neutrophils continues to be so great that they are being called out from the bone marrow even though immature. When the count shows that the percentage of such cells is returning to normal, the condition is styled a shift to the right. It suggests that the emergency is over and that the demand is decreasing.

**Eosinophiles.**—These have somehow become specialized along another path. Grafted upon a similar background of organization is a different structure and function. Their characteristic cytoplasmic granules and nuclei have been mentioned. Their private lives are even more beyond our ken than those of the neutrophils. Like the neutrophils these eosinophilic cells are of marrow origin, but comparatively few of them are found in the blood stream. Their primary function is not phagocytosis, but they can ingest bacteria (Hertzog, 1938, Fig. 11).

It is in the tissue fluids that eosinophiles are chiefly seen. A survey of these environments shows that, while they may occur in practically all of them, they are ordinarily most numerous in the extravascular fluids under the linings of the respira-



tory and digestive tracts. With respect to inhaled substances and to materials taken in by mouth these are very exposed positions.

The members of the class who have high eosinophile counts often give a history of asthma or of being allergic to certain foods. It is possible also that they may be suffering from a mild infection with parasites acquired by eating incompletely cooked hamburger that has been made into a more generous helping by addition of pork which is cheaper than beef. Be this as it may, an eosinophilia in the blood where it is easily seen and in the subepithelial tissue fluids where it is overlooked unless revealed by examination of tissues removed at operation or autopsy, appears in many cases to be related to exposure to a wide variety of materials to which the individual is unduly sensitive. But other factors may be involved. It is remarkable how quickly an increase in blood eosinophiles can be produced experimentally.



FIG. 11.—Eosinophiles containing phagocytized streptococci from a case of eosinophilia  $\times 1500$  (From Hertzog, Courtesy of Am. J. Path.)

Chillingworth *et al.* (1933-34) selected 15 medical students and handicapped expiration by using a partly blocked shutter valve. Counts made beforehand showed an average of 0.43 per cent eosinophiles. During the period of dyspnea which averaged two and one-quarter minutes, other counts revealed an average of 7.19 per cent. Five minutes later the eosinophilia had subsided to an average of 1.1 per cent. Others have found that more radical interference with expiration in rabbits and dogs produces a more pronounced eosinophilia. In these cases the air sacs are distended and the visceral sensory fibers of the vagus are undoubtedly stimulated. And we recall that Hajos and associates (1926) secured marked eosinophilia by direct electrical stimulation of the vagus in dogs. The average rise spread over 10 animals was from 8.1 to 10.5 per cent. Chillingworth believes that eosinophiles are released from the bone marrow by a selective nervous reflex. Nevertheless, he and others are fully aware that all of these procedures are accompanied by severe asphyxia (increase in  $\text{CO}_2$  tension of blood).

Turning to the tissue fluids, Nemours (1933) evoked an eosinophilia of maxillary sinus mucosa by giving pilocarpine which attained a maximum in five minutes and disappeared after twenty-four hours.

The results of these experiments seem to indicate a change in distribution of eosinophiles rather than an increase in production, though the latter might happen if stimulation were sufficiently prolonged. Some think that eosinophiles serve as detoxifying agents. It is, of course, conceivable that they secrete some substance which combines with the offending material to render it innocuous. Another possibility is that they may adsorb or ingest the material and thus tuck it away where it will do no harm.

**Basophiles.**—These cells have perfectly definite structural features faintly staining, rounded or slightly indented nuclei, and basophilic cytoplasmic granules which are of irregular size and seldom so densely packed as in eosinophiles or neutrophiles.

The percentage of basophiles is so small as to seem almost negligible. Moreover any considerable increase in health or disease is conspicuous by its absence. But in some amphibia the blood basophiles are more numerous than the finely granular leucocytes which correspond to human neutrophiles. The obvious conclusion is that production of basophiles is a vestigial phenomenon comparable in a small way to the continued development of a useless appendix.

But this interpretation may not be correct. What gives us pause is the chance of a relationship between these cells and extravascular, or tissue, basophiles of which more later (p 274). Suffice it to say here, though the tissue basophiles are a much more robust tribe, they do show a structural resemblance to those in the blood. The blood basophiles, like the neutrophiles and eosinophiles originate in the bone marrow and are discharged into the blood. They are feebly mobile. Perhaps they invade the tissues, also like their associates, and take on a new lease of life. The claim has been made that tissue basophiles produce heparin (Jorpes, Hölmgren, and Wilander, 1937). It would seem the part of wisdom to investigate the relation, if any, between the blood basophiles of forms that possess them in abundance and heparin production.

Before passing to the *non-granular* leucocytes a brief review of the common properties of the already described granular leucocytes is in order. All three varieties originate in the bone marrow and are therefore said to belong to the myeloid series (*Myelon*, marrow + *eidos*, like). The word "series" is here used to include cells of marrow origin, that may or may not enter the blood stream, in addition to the granular leucocytes which are finished products of the marrow. As postmitotics the granular leucocytes serve their purpose, if the opportunity is given, and die without offspring. They all contain specific granules by which each can be conveniently recognized. Another common feature is that they give a strongly positive reaction for peroxylase. This is of clinical use, because, by contrast, the non-granular leucocytes are negative or very feebly positive. When, therefore, atypical white cells are encountered, which are rich in peroxylase, the possibility that they are the granular leucocytes or their precursors is more seriously entertained.

The *peroxylase reaction* is simple and should be tried

- 1 Dry blood smears in air.
- 2 Flood the slides with solution A (0.5 per cent copper sulphate). After one minute pour off the solution but do not wash or dry the slides.
- 3 Apply solution B (rub up in a mortar 0.2 gm benzidine with a few drops of distilled water. Then add 200 cc of distilled water and filter. To the filtrate add 4 drops of 3 per cent hydrogen peroxide and keep the resulting solution in a dark bottle). Allow to act for two minutes. Then wash in tap water.

4 Stain with solution C (10 per cent safranin in distilled water) for one minute Wash again in tap water and dry

The peroxidase granules are colored blue and the nuclei orange red while the specific cytoplasmic granules remain unstained (See Technique p 104)

**Lymphocytes**—For convenience lymphocytes are arbitrarily classed in 3 categories

(1) *Small*, diameter equal to 1 erythrocyte or less—these are the most numerous those ordinarily found in the blood and intended when the term lymphocyte is employed without qualification

(2) *Intermediate* diameter greater than 1 erythrocyte and less than 3—occur sparsely in blood and abundantly in lymphoid tissues

(3) *Large* diameter greater than 3 erythrocytes—normally confined to lymphoid tissue and bone marrow and only entering blood stream in pathological conditions

The principal structural features of a small, or typical lymphocyte is its spherical or oval nucleus and its small amount of cytoplasm often visible on only one side Indeed the nucleocytoplasmic ratio may be higher than that of any other kind of cell in the body The nucleus stains intensely with basic dyes and is rich in chromatin The cytoplasm is basophilic and contains a large amount of mitochondria in proportion to its volume as well as a variable number of curious granules called azurophilic for in smears they are colored by the azure component of the mixture



FIG. 12.—Lymphocyte of bovine dividing under stimulus of a protozoan parasite *Theileria parva*. Parasites are seen just above the chromosomes (Cowdry and Danks Parasitology)

Events in the lives of small lymphocytes are but little known to us They originate by mitotic division of parent cells of intermediate size in the lymphatic tissues Lymph enters the venous blood by two channels a large thoracic duct and a small right jugular lymphatic duct Therefore the number of lymphocytes dumped into the blood in twenty four hours can be estimated far more accurately than the number of neutrophils which are swept into the blood in many streams from the bone marrows Since this number exceeds the total lymphocytes present in the circulation at any one time which remains fairly constant and evidence is lacking of their death or wholesale change into other cells it follows that most of these cells remain

in the blood stream less than twenty four hours In the rabbit lymphocytes in the circulation are replaced as quickly as five times a day (Sanders Florey and Barnes 1940)

During this brief period the lymphocytes seem to do nothing more than passively to drift around Mitoses are practically never seen except when there is some unusual stimulus to cell division (Fig. 12) Nuclear division suggestive of amitosis have been described (Fig. 13) but we are not satisfied that this splitting of nucleus is followed by division of cytoplasm to produce 2 new individuals either here or in any other part of the body unless some instances of division of the whole cells are actually seen

What calls so many lymphocytes from the vascular lumina out into the tissue fluids has not been discovered But normally the call is insistent along the digestive

tract and negligible from the vessels in the extremities. A massive migration of blood lymphocytes into the tissue fluid just within the epithelial linings of the stomach and intestines may take place in fasting. A Negro executed in Clayton showed this beautifully (Fig 130). He had lost his appetite forty-eight hours before death. In pathological states invasion of tissue fluids can occur in any situation. Lymphocytes arrive after the neutrophils and stay much longer. They may arrange themselves diffusely or line up as perivascular "collars."

Having thus returned to the tissue fluids, after travelling away in the lymph and circulating in the blood, the careers of these individual lymphocytes continue to show lack of uniformity and the element of chance. Some die, but not many, because signs of degenerating lymphocytes are seldom encountered. Others escape into the intestinal lumen and die there. Still others stay put and end their lives by mitotic division in the production of offspring.



FIG 13 —Stages in amitosis of lymphocytes. Blood film of a mouse six days after an exposure to heat, showing various stages of amitosis in the lymphocytes. (Redrawn from Murphy and Sturm, J Exper Med)

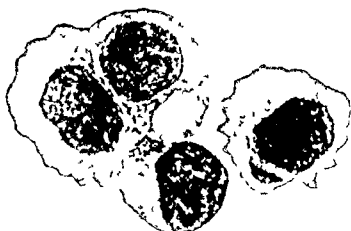
The relation of extravascular lymphocytes to the other non-granular white inhabitants of tissue fluids is much debated. It is possible that they enlarge to form intermediate and large lymphocytes. Whether some normally enjoy a new lease of life as monocytes, plasma cells or Russell body cells (see connective tissues) may be determined at some time in the remote future. Given an adequate stimulus, such as the subcutaneous injection of egg white, Kolouch (1939) found that within a very short time cells can be seen which seem to be transitional between small lymphocytes and monocytes (macrophages) as is illustrated in figure 14. In groups they seem to rise to the occasion and swell. Lymphocytes can also phagocytize bacteria (Fig 15) but this seldom happens and certainly is not their chief function. Evidence has been presented that they play some part in resistance to experimental cancer and tuberculosis in mice (Murphy *et al.* 1919). Lymphocytes are often increased in convalescence from acute infections and in chronic conditions of many sorts. They are very susceptible to x-ray. They are further discussed in connection with the lymphatic system which is more their home than the blood.

**Monocytes.**—These differ structurally from most lymphocytes seen in the blood. They are larger, have a less spherical nucleus which stains less intensely (Fig. 7),

possess more cytoplasm which is less basophilic and contains more neutral red granules often disposed in rosette formation in the nuclear concavity and they are relatively less numerous than the lymphocytes



14 hours after injection



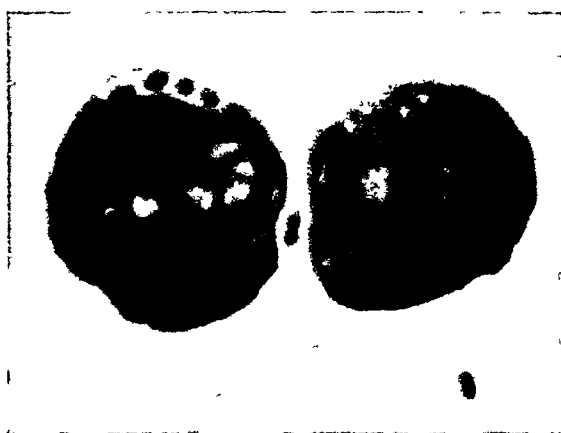
18 hours



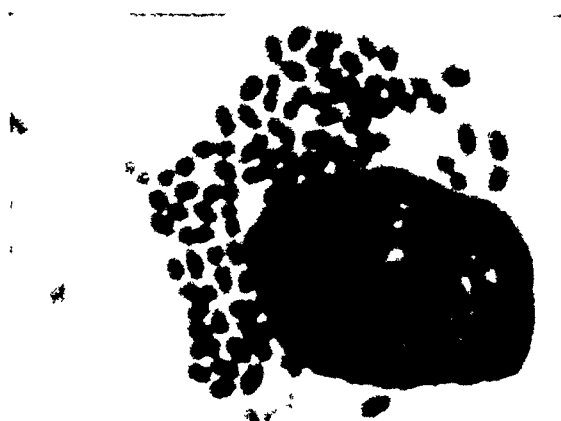
23 hours

FIG 14 - Lymphocytes and macrophages in tissue spreads stained by May-Grünwald Giemsa method obtained by biopsy from rabbits at various intervals after subcutaneous injection of egg white. Shows lymphocyte macrophage transformation.  $\times 1500$  (Holouch courtesy of Am J Path)

Identification of monocytes in normal blood is usually feasible but it requires practice. Yet when many non-granular cells are present especially in pathological



*Small lymphocytes*



*Large mature lymphocyte*



*Monocyte*

FIG 15 — Small lymphocytes from a case of lymphatic leukemia showing phagocytoses of streptococci.  $\times 1500$  Large mature lymphocyte from a case of infectious mononucleosis also showing phagocytosis of streptococci  $\times 1800$  Monocyte from myelogenous leucemia—phagocytosis of staphylococci  $\times 1800$  (From Hertzog, courtesy of Am J Path )

conditions it is not possible always to decide whether a particular cell is a monocyte or a lymphocyte. A series of cells can be selected ranging from a typical lymphocyte on the one hand to a typical monocyte on the other in which one cannot be sure where the lymphocytes end and the monocytes begin.

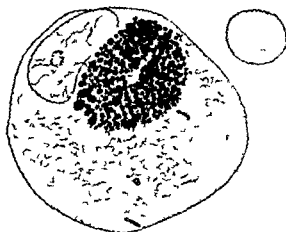


FIG. 16.—Photograph of a drawing of a monocyte which has phagocytosed two tubercle bacilli which are represented unstained and which were unquestionably still alive. Size may be estimated from the accompanying representation of a red blood cell. (Photograph by R. S. Cunningham.)



FIG. 17.—Response of monocytes to leprosy bacilli: A, Small lymphocyte as a measure of size. B, Monocyte containing a mass of bacilli (represented in black) multiplying in a more fluid part of the cytoplasm. C, A larger monocyte with several groups of multiplying bacilli. D and E, Cells in which the bacilli are granular and degenerating. The clear circles in the cytoplasm represent droplets of fat.  $\times 950$

How is such a series to be interpreted in attempts to conjure up a picture of the life of an individual monocyte? It may mean that a cell born and bred as a lymphocyte can enlarge, undergo structural reorganization and change bodily into a monocyte. If so this cell lives first as a lymphocyte and then as a monocyte. Unfortunately, however, the series is made up of unknowns arbitrarily selected because at the moment of observation each cell considered intermediate differed from its neighbor in being a little less like a typical lymphocyte and more like a typical

monocyte. There is no means of knowing how each of them would have aged, whether the resemblance to a monocyte would have increased or decreased. When Ebert, Sanders and Florey (1940) held individual lymphocytes under observation for twenty-four hours in transparent chambers inserted in the ears of rabbits, the said lymphocytes continued to exhibit typical movements and showed no signs of changing into any other type of cell. This does not however signify that lymphocytes never change into monocytes. But what would happen over a longer time and under different conditions is pure speculation. Because there are so many lymphocytes and so few monocytes, the transformation, if it occurs, can involve only a very small number of lymphocytes.

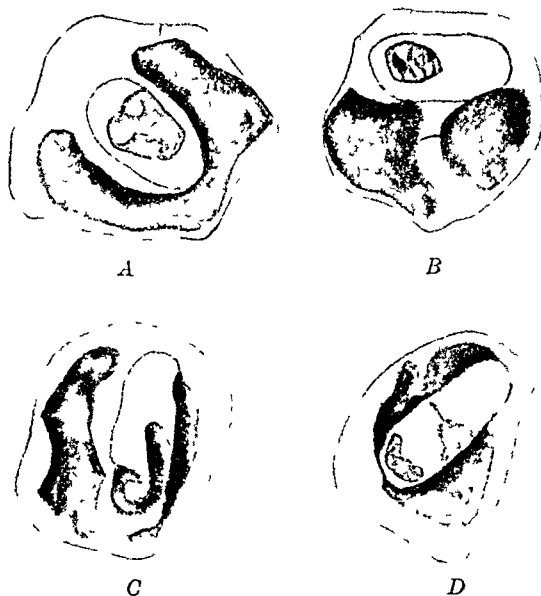


FIG 18 —Illustration showing parasites within monocytes. A and B, *Hepatozoon muris* in the rat, D and C, *Hepatozoon canis* in the dog.  $\times 3000$  (Redrawn from Wenyon, Protozoology, Williams & Wilkins Company)

Another possibility is that when a lymphocyte divides, for some obscure reason and in a small minority of cases, one, or both, of the daughter cells grows up as monocyte. Or monocytes may conceivably develop, not from lymphocytes in either of these two ways, but from some other sort of relatively undifferentiated cell by transformation or by mitosis. Once formed, some monocytes unquestionably live intermitotic lives and by division give rise to other monocytes in which case our individual monocyte begins life as a monocyte.

The subsequent stages in its existence depend on chance. Whether in the blood, or in the extravascular tissue fluids in which they probably originate and to which they are prone to return, monocytes exhibit the faculty of being able to pick up materials of many kinds with which they come in contact. Monocytes indeed have been styled the gypsies of the cellular community. In their behavior they differ sharply from lymphocytes, which wander about as much as they do but without having particles stick to them.

Monocytes also differ from neutrophils in their phagocytic properties. This is a long story only part of which can be told. Both, for instance, take up the bacilli of tuberculosis and leprosy when exposed to them. But in the monocytes some of the organisms continue to live (Figs 16 and 17), and in leprosy multiply within the



cells so that the latter become enormously distended, whereas the neutrophils either kill the organisms or are killed by them and never swell up. Not only these bacilli but protozoan parasites can live within the monocytes—witness the hepatozon (Fig. 18). Parasites that can live within lymphocytes are still more numerous. Neutrophils generally destroy what living organisms they ingest. A wide variety of foreign materials and cellular debris are taken in by both monocytes and neutrophils. Figure 19 indicates the response of monocytes to carbon. Again there is great enlargement, not present in the case of neutrophils.

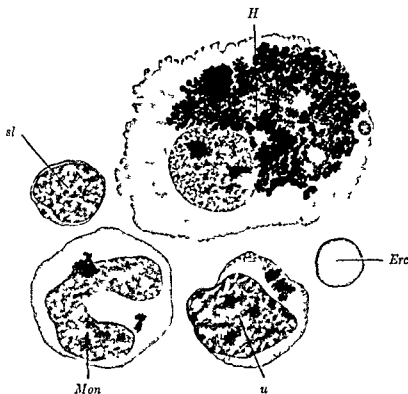


FIG. 19.—Dry smear of blood from right ventricle of rabbit after repeated intravenous injections of saccharated iron oxide and India ink. *H*, Macrophage; *Mon*, monocyte; *u*, transitional cell between monocyte and lymphocyte; *sl*, lymphocyte; and *erc*, red blood cell to serve as standard of size. (Maximow, Cowdry's Special Cytology, Paul B. Hoeber Inc.)

This monocytic enlargement supplies another series which begins with a typical monocyte and arbitrarily ends with a typical macrophage—a cell so large that it is ordinarily filtered out by the first capillary bed encountered and consequently appears but rarely in the blood stream. One cannot say where the monocyte ends and the macrophage begins, but all are not agreed as to the composition of the series. Some think that it is made up of at least 2 types of cells which respond in the same way to particulate matter; that macrophages are not enlarged monocytes.

The monocytes that become so greatly distended probably go to pieces. Their lives are therefore postmitotic insofar that they extend from the mitosis which gave them birth to death and not to another mitosis. But a great many never have a chance to become phagocytic. Perhaps the majority of these are intermitotics with individual lives limited by the next following mitosis and do have the



FIG. 20 — Illustrating the behavior of chicken leucocytes in tissue cultures as seen after supravital staining with neutral red and janus green showing neutral red granules and mitochondria. 1, Polymorphonuclear leucocyte two hours in plasma and embryo juice; 2, erythrocyte for comparison, 3, blood monocyte two hours in plasma and embryo juice, 4, same, but different culture. 5, blood monocyte twenty-four hours in plasma and embryo juice, 6, same, forty-eight hours, 7, same, seventy-two hours, 8, same, ninety-six hours. 9 and 10, tissue macrophages twenty-four hours in plasma and embryo juice. (Carrel and Ebeling, courtesy of J. Exper. Med.)

potentiality of forming other cells a property not shared by the definitely post mitotic neutrophils eosinophiles and basophiles. This brings up one last great difference between non granular and granular leucocytes. The former (lymphocytes and monocytes) can become malignant as in Hodgkin's disease and some leucemias. In no other group of cells are the relationships so little known. The division of hematologists into sects is to be primarily attributed to the haziness of ideas concerning non granular leucocytes. Some believe this, others believe that, there are the unitarians dualists and trileists, who conceive of blood cells as coming from a single type from two types and from three types of cells, the "extreme unitarians and the neo unitarians." To mention them by name, and to attribute the wrong belief to one of them would be unpardonable.

However some of the transformations of non granular leucocytes that one must bear in mind as possibly occurring are indicated in figure 1. The problem will unfold a little with consideration of lymphatic and connective tissues.

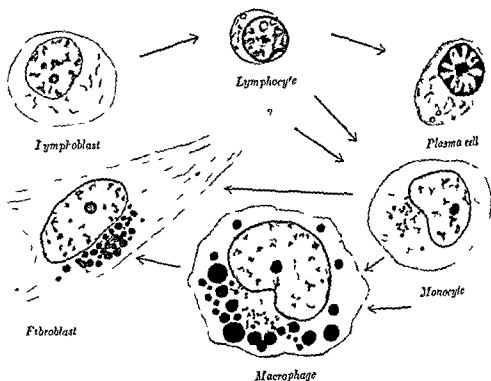


FIG. 21 Some possible interrelations of non granular leucocytes

### SUMMARY

Knowledge of function lags far behind knowledge of the structure of leucocytes. We think at once of phagocytosis as a very useful property. Two kinds of leucocytes lead all other cells of the body in this duty. Both neutrophils and monocytes can ingest living bacteria as well as dead particles of many kinds, but there is a sort of division of labor between them. The neutrophils are the first on the spot to combat bacterial invasions of the tissues and they are most efficient in the destruction of the bacteria they phagocytize. The monocytes mobilize more slowly, lead in the cleaning up of foreign material, dead cells and parts of cells, and become greatly

distended by the ingested material. Phagocytosis by other kinds of leucocytes is unusual and evidently not their main function.

Motility is a feature of all 5 types and, like structure, is characteristic of each. But merely to move about is not a service to the cellular community. Position is not a good clue to function. Yet the manner in which the eosinophiles arrange themselves in the most exposed tissue fluid environments beneath the epithelial linings of the respiratory and alimentary tracts, and their increase in number in response to foreign materials to which the body is unduly sensitive, suggests a rôle crudely expressed by the term *detoxification*.

It has recently been claimed that the basophiles produce *heparin* but this remains to be proved. Lymphocytes are the outstanding enigmas. Like the monocytes, and unlike the granular leucocytes, they are at least potentially intermitotics. They may serve, not only in their own right as lymphocytes but also as producers of other cells. But these other cells (monocytes, plasma cells and Russell body cells) are so few in number compared with lymphocytes that the vast majority of lymphocytes cannot be expected to produce them.

## CHAPTER II

### RED CELLS AND OTHER FORMED BODIES

**Red Cells** — These elastic, biconcave lens-shaped hemoglobin containing bodies are of fairly uniform size. In dried smears their diameter is usually placed at  $7.6\ \mu$ . This figure should be remembered because it is convenient to estimate the size of other cells in smears by reference to erythrocytes. Moreover the girth of capillaries is adjusted so as to let them through easily. Figure 22 is instructive because it gives an idea of how different must be the capillary circulation in forms possessed of large erythrocytes.

Like the granular leucocytes these cells are produced in the bone marrow, but in their development Nature has produced cells intended for service after their death. Seen in the circulation red cells are simply hemoglobin containing bodies

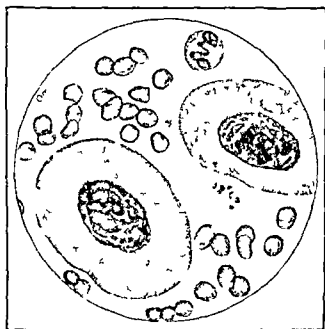


FIG. 22 — Giant nucleated erythrocytes of tailed amphibian (*Amphiuma*) compared with human ones. Wright's stain  $\times 960$ . (From Wintrobe Clinical Hematology, Lea & Febiger.)

with elastic walls. In the circulation of gold and silver mankind has discovered after many trials that these precious metals should be coined in the shape of circular discs. Red cells are like coins except that their sides are slightly hollowed out to form biconcave lens-shaped structures. They stack up like coins in *rouleaux* when rather thick mounts are made of fresh blood. But they are obviously different from coins because their surfaces are soft and yielding. Otherwise they would damage the walls of the vessels and be quickly broken up themselves.

It is sufficient to watch them being rushed along in the web of a frog's foot, or in some other convenient material to see how readily they change their shape only to resume it again equally promptly. This speaks for truly remarkable elasticity and

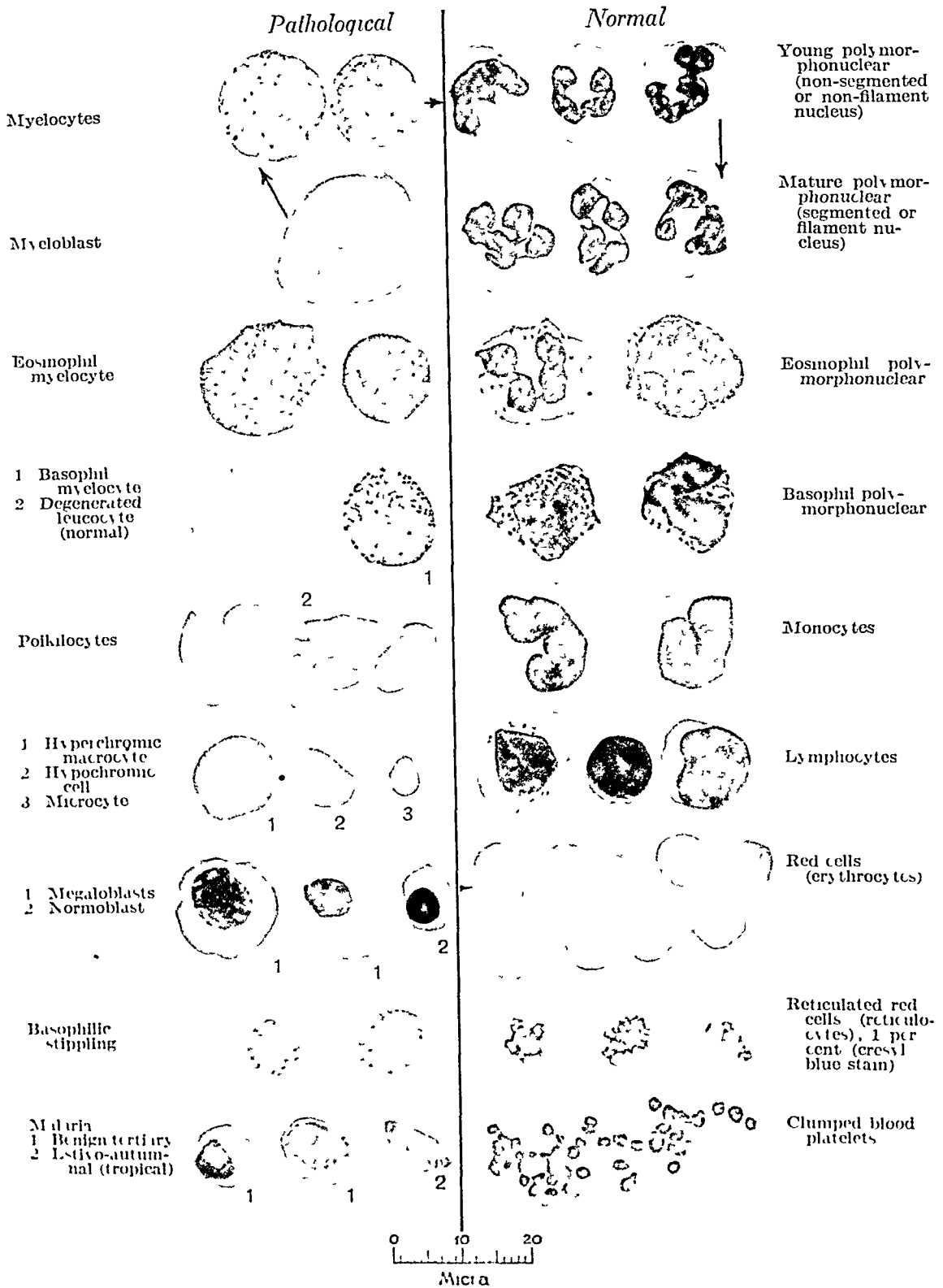


FIG. 23 — Normal and pathological blood cells. Wright's stain  $\times 1000$  (Nicholson, Laboratory Medicine)

organization. Living cells could not stand the racket so long. If it is not feasible actually to demonstrate this deformation and recovery, the process can be clearly seen in a moving picture film entitled 'Micromanipulative Studies of Blood Capillaries' prepared by Dr B W Zweifach, Dept of Biology, New York University, Washington Square College.

Despite this resistance to trauma red cells respond easily to physical and chemical changes in their environment. It is a difficult matter to make a mount of fresh blood in such a way that they are not distorted. The plasma of the circulating blood is said to be *isotonic* because in it the reds retain their normal appearance. Approximately isotonic fluids can be made for use when the blood is studied outside the body. Among these may be mentioned Ringer's solution and a solution of 0.85 per cent of sodium chloride in distilled water. Fluids which contain too much salt are referred to as *hypertonic* and those with less than the right amount of salt as *hypotonic*.

It is an interesting experiment to watch directly the effects of such solutions on red cells. A small amount of blood from the finger tip is mounted on a slide. A drop of hypertonic (2 per cent) salt solution is placed along one edge of the cover slip in contact with the blood. The solution mixes slowly with the blood and actual stages in the shrinkage (crenation) of the reds can be followed. If it is desired to hasten the process the solution can be drawn through by touching a piece of blotting paper to the blood along the opposite edge of the cover. Shrinkage results because substances in solution tend to become evenly distributed. The salts which are present in the hypertonic solution in a higher concentration than in the plasma to which the cells had been accustomed, will diffuse into the cells in an attempt to become evenly distributed and to reestablish osmotic equilibrium. At the same time water will leave the cell, tending to reduce the concentration of salts in the surrounding fluid. The abstraction of water inevitably results in a marked decrease in volume of the cells. When a hypotonic solution of say 0.2 per cent of sodium chloride is used in the same way the reverse happens again as the result of the tendency of substances in solution to become evenly distributed although the actual shift is very slight indeed. Salts leave the cells to increase the concentration without and water enters leading to decrease in concentration, increase in volume and great swelling of the cells so that they even may burst.

No adequate conception of red cells can be obtained by consideration only of their dead remains. Their life history, to the best of our knowledge today is divisible into three periods.

(1) *Normoblastic*—Each and every red blood cell originates in bone marrow by mitotic division of its parent in the hemoglobin production line. It is born charged with a certain amount of hemoglobin and a nucleus and is known as a *normoblast* (Figs 23 and 24). Since it is destined for service, not for multiplication, classification as a postmitotic is justified. This normoblastic stage of its existence is normally passed in the marrow. The nucleus disappears. In *erythroblastosis fetalis* (Reisner 1943) many instances are encountered of breaking up of the nucleus by budding. Some of the parts thrown off persist for a time as the Howell-Jolly bodies. See Schleicher (1942) on the nature of Cabot rings. There remains in the cytoplasm a considerable amount of material which is stainable as a kind of reticulum with neutral red, brilliant cresyl blue and similar dyes.

(2) *Reticuloeytic*. In this second stage the cell is called a *reticulocyte* (Fig. 25) and there is reason to think that it is still partly alive because masses of red blood cells including many reticulocytes consume oxygen. Such cells ordinarily remain residents of the bone marrow. At birth a considerable percentage of reds in the circulation may be in this part of their life cycle though in adults the reticulocytes

gaining entry to the blood stream are only about 1 per cent of the total number present.

To study reticulocytes select a 7 to 8 lb rabbit and begin the experiment taking 25 cc. of blood and a like amount every second day including the late afternoon of the tenth day. The best way is to shave a fairly large area of the thoracic wall on the left side. Locate the point of maximum cardiac pulsation by feeling between the 3rd and 5th ribs. Treat the skin with alcohol. Insert vertically a sterilized hypodermic needle attached to a sterilized syringe of large capacity. When the ventricle is punctured blood surges up in the syringe. (The messy way, harder on the rabbit and more likely to cause infections, is to bleed from the ear vein.) During the tenth day prepare sufficient slides for the class. Place on each slide near the end 1 drop of 1 per cent brilliant cresyl blue in absolute ethyl alcohol. With the end of another slide draw the fluid over the slides and allow it to evaporate. In the morning of the eleventh day give the slides to the students, bring in the rabbit with shaved ear, make a small puncture and have the students smear blood as already described on the prepared surfaces of the slides, dry in air and examine. Reticulocytes are seen to be greatly increased in number and are readily identifiable by the purplish-blue color of the reticular material in them (Technique p. 171)

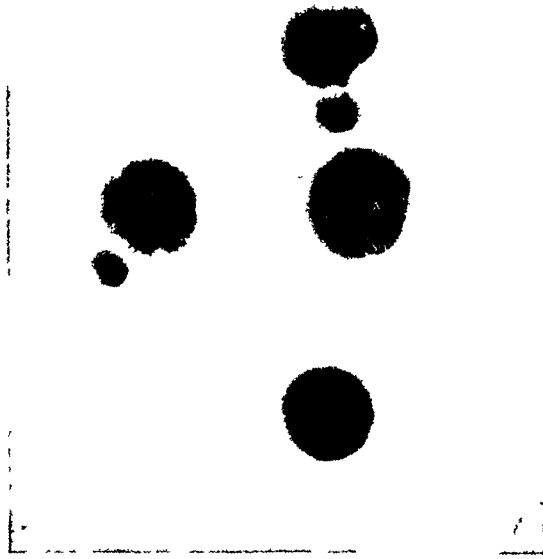


FIG 24 —Nuclei of normoblasts budding off Howell-Jolly bodies. Blood smear from case of erythroblastosis fetalis in a two-day old female. Colored by Wright's stain and counterstained by Giemsa's.  $\times 1800$  (Lent by Dr. Carl V. Moore)

(3) **Erythrocytic.**—With disappearance of the reticulum, usually completely accomplished in the bone marrow, the cell becomes an *erythrocyte* and is washed into the blood stream. As already stated, however, a few enter in the reticulocytic phase. Eventually the erythrocyte wears out and is removed from the circulation. The total existence span of a red blood cell, during which it is called these three names, is not accurately known. Several methods of estimations are employed. The newest one is to tag the erythrocytes with radioactive iron (Cruz *et al* 1941). When exposed to this iron the erythrocytes take it in and the time that some of them remain radioactive can be measured. There is reason to think that the ter-



mental phase of usefulness in death for many is about fifty days and for a few much longer perhaps one hundred days or more. See also Graam (1942)

The ultimate fate of the red cells is of interest. Some think that they break up in the blood stream presumably when their colloids have through advancing age lost their remarkable elasticity and for this reason can no longer stand the racket. But the uniformity in size and shape of red cells in the circulation is impressive and fragments are so rare as to cause comment whenever they are seen. Reds are not ordinarily phagocytized by non granular leucocytes in the blood stream. Many are somehow squeezed through the walls of the venous sinuses of the spleen. In the extravascular tissue fluid of this organ they are engulfed and digested by micro-

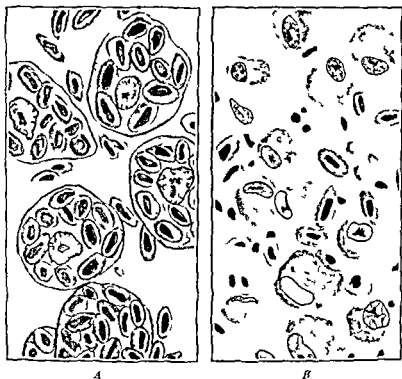


FIG 20-1 Phagocytes of lung culture from an immunized rabbit filled with erythrocytes five days *in vitro* erythrocytes added twenty four hours previously. B Normal lung culture phagocytes surrounded by erythrocytes no evidence of phagocytosis four days *in vitro* erythrocytes added twenty hours before fixation (Redrawn and modified from Bloom Arch Path and Lab Med)

phages. Others come in contact with the endothelial cells lining the sinusoidal capillaries of the liver where the current is slow and are taken in by them. These two are the main sites of disposal but wherever leucocytes of this sort encounter very old reds they are likely to phagocytize them. Why less old but definitely dead reds are declined by the phagocytes remains to be discovered. Perhaps with advanced age their surface becomes more acceptable. As Bloom (1927) proved immunological factors can operate. Thus the phagocytes of normal rabbits are apparently unable to cope with erythrocytes of pigeons but when the rabbit is immunized against them the phagocytes actively ingest them (Fig 20). Whatever the means employed the red blood cells are removed from circulation while they

still possess considerable hemoglobin and presumably retain ability to function in tissue respiration. Useless reds are not allowed to clutter up the circulation.

The service of erythrocytes is fairly well understood. Why the hemoglobin should be locked up in their interior is an interesting question. Stated in simple terms the answer is twofold. The properties of the capillary walls are such that if the hemoglobin were free in the plasma it would pass through them and leave the circulation. This is what actually happens when red cells are laked (dissolved) in the blood stream, the liberated hemoglobin immediately finding its way out by the kidneys. To hold free hemoglobin would involve the construction of capillary walls which would retain not only hemoglobin but many other essential substances. In the second place, the shutting up of hemoglobin within the red cells has supplied a protected fluid medium of constantly regulated physical properties in which the hemoglobin can function far more efficiently than in the blood stream where the high concentration of sodium chloride would be a deterrent. As Barcroft (1922) says:

"In the interior of the red blood corpuscle the haemoglobin exists in a world of its own, by this device Nature has at a stroke increased the efficiency both of the blood and of the haemoglobin, it has saved the blood from possessing physical properties which the haemoglobin would otherwise have conferred upon it—increased viscosity, and an impossible osmotic pressure, it has saved the haemoglobin from being dissolved in a solvent (NaCl) of other than maximal efficiency and from functioning at a hydrogen-ion concentration in which the remarkable properties of haemoglobin as a respiratory pigment would not betray their maximal efficiency—such is the *raison d'être* of the red blood corpuscle."

Data on the chemical structure of erythrocytes are conveniently reviewed by Williams, Erickson and Macy (1941). The course of iron through the body and its rôle with copper in hemoglobin synthesis is clearly presented by C. V. Moore (1939).

Counts of erythrocytes supply valuable information but experience in making them must be left to the course in clinical microscopy. In the examination of smears of his own blood and of those of his neighbors the student should learn three things: (1) to appreciate the alterations in form and staining reactions that are due to faulty technique and are not attributable to conditions actually existing *in vivo*; (2) to gain an impression of the usual degree of uniformity in the appearance of erythrocytes and (3) to be able to identify red blood cells which are prematurely discharged into the circulation.

**Platelets.**—These can be seen in circulating blood when the vessels are observed at fairly high magnification. Each is a distinct individual with sharp outlines. However, when examined in fresh mounts, they do stick together and in stained smears they are usually more or less clumped. Platelets are ordinarily much smaller than any blood cells and it is possible always to see that they are made up of at least two components: a clear ground substance with granules in it. That they are cytoplasmic fragments is evident. The most uniformly present granules bear a striking resemblance to the cytoplasmic granules of megakaryocytes (large nucleated cells) of the bone marrow in their coloration by the stains of Wright and Giemsa. Other granules can be supravitality stained with janus green and appear to be mitochondria.

Figure 26 shows stages in the breaking off of platelets in which granules resembling those of megakaryocytes are conspicuous. But it is in the bone marrow rather than in the blood stream that this takes place and it would be unwise to conclude

that megakaryocytes are the only source of platelets. Besides the bone marrow they seem to come from the spleen.

A point always to be borne in mind in the morphological study of platelets is that they are subject to great variation. In blood during active regeneration platelets 25 to 50  $\mu$  in length have been reported (Tocantins 1938). In blood that has been prevented from coagulating by the addition of oxalate or citrate solutions the platelets may extend spine-like processes. Sometimes platelets look like microorganisms.

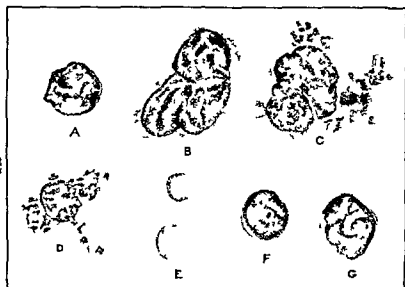


FIG. 20.—Megakaryocytes in the peripheral circulation in a case of myelogenous leucemia compared with red cells and myeloblasts. *A* Naked nucleus of megakaryocyte. *B* megakaryocyte with cytoplasm disintegrating from same case. *C* and *D*, megakaryocytes whose cytoplasm is breaking up and giving rise to platelets. *E* showing variation in size of red. *F* and *G* myeloblasts. (Redrawn from Minot, *J. Exper. Med.*)

It is a helpful exercise for the student to compare the platelets (as well as the blood cells) in thick and thin smears of his own blood made (1) when absolutely fresh (2) after it has exuded from the needle puncture and has remained on the surface of the finger for one minute and (3) one hour after mixing on the slide with 2 per cent aqueous potassium oxalate. A demonstration should be made for his benefit of platelets and fibrin viewed by the dark field microscope.

To determine roughly whether platelets are present in approximately the normal number prick the alcohol sterilized finger through a drop of the diluting fluid of Rees and Ecker (38 per cent sodium citrate, 0.2 per cent commercial formalin and 0.1 per cent brilliant cresyl blue). Mount and count quickly the number of platelets per erythrocyte. If there is about 1 platelet per every 20 erythrocytes and the total count of erythrocytes is that considered usual for adult males, namely 5 000 000 per c mm (which is a fair preliminary supposition) then the number of platelets is about 250 000 c mm. This is considered normal. In case the ratio of platelet to erythrocyte (checked by the instructor) is more than 1:10 or less than 1:40 refer the student to the Student Health Service (Technique, p. 160).

Attempts have been made without much success to relate the structural features of platelets to their age and physiological activity. It is known that some platelets consume oxygen and are presumably living. How long they go on living is a question that has not been answered. Tocantins (1938) in his extensive review, gives much information. It appears that all platelets in the circulation can be

replaced in three to five days, that 100,000 per c.mm. can be formed each day and that in certain pathological states the platelet count can rise to 1,000,000 per c mm in the short space of twenty-four hours. Worn out platelets are destroyed by phagocytic action chiefly in the great blood filters, the spleen and the liver. The platelet turn over is evidently fairly rapid. That they play an important rôle in blood coagulation is assured. Nature not only makes dead erythrocytes useful, but also cell fragments, in the form of platelets, and regulates the fragmentation so closely that counts of the pieces are of clinical significance.

**Chylomicrons.**—This name is given to tiny droplets of fat, visible in the blood plasma after a fatty meal by light reflected from their surfaces when examined in the dark field. The prefix, *chylos* (G for chyle), is appropriate, because they enter the blood in the lymph, or chyle, having come from the area of intestinal absorption, and the suffix *micron* (G for minute body) is likewise fitting, because of their small

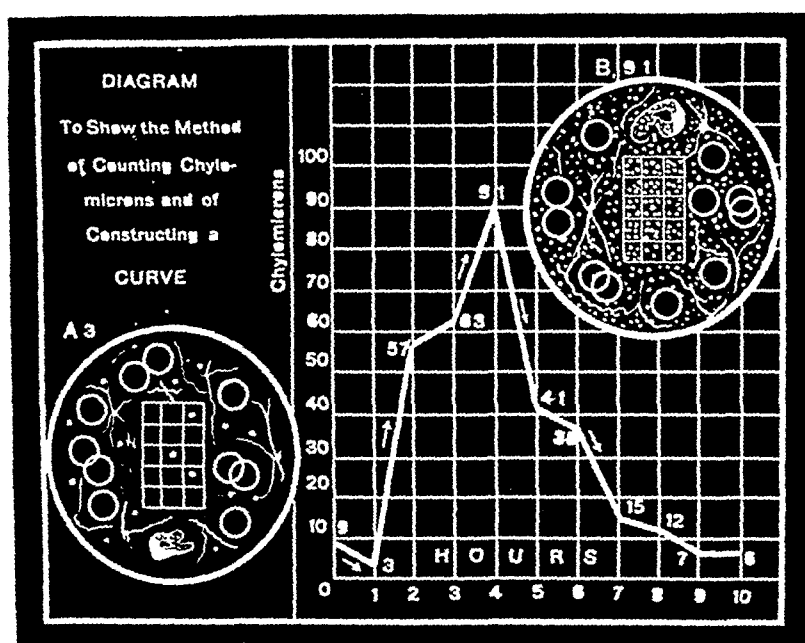


FIG 27.—Diagram illustrating method of counting chylomicrons and of constructing a curve. (Gage and Fish, *Am J. Anat.*)

size. Chylomicrons have been thoroughly studied by Gage and Fish (1924-25) whose paper should be read carefully by every medical student. Evidence has been provided by Ludlum, Taft and Nugent (1931) that the fat is encased in a protective film of protein which may condition its utilization. The distribution of microscopically visible fat *via* the blood stream is an important factor in metabolism and will be referred to again in the description of fatty tissues (p 277). Owing to the fact that "fat is metabolized and deposited more quickly during the day than during the night" a rhythm exists in chylomicron frequency (Bohm, *et al*, 1941).

Figure 27 illustrates the method of making a chylomicron curve. The circle A represents a portion of a preparation of blood, taken before a fatty meal, seen in reflected light. The 10 smaller circles within it indicate red blood cells, the filaments, fibrin; and the small white spherules, chylomicrons. Three of the latter are represented as within the space covered by a ruled disc inserted in the ocular.

The other large circle B shows the condition of the blood four hours after a fatty meal when 91 chylomicrons were within the same space

Select 2 student volunteers Have the first take for breakfast at 8 A M cereal with a pint of cream (or if this is not available a quart of milk) and the second bread and a bottle of Coca Cola Between 11 A M and 12 noon make a mount of the blood of each and examine in the dark field (Technique p 52)

**Other Particles**—Having in mind the variety of blood cells and their ceaseless circulation often crashing against the walls of the vessels, it is surprising that other particulate matter is of such rare occurrence In stained smears one occasionally finds a few small bodies known as *hemoconia* (*G haemia* blood + *konis* dust) The term is loosely used and not all of the particles thus designated are of the same nature Some may be cell debris others coagulated blood protein dye particles or atmospheric dust that has settled on the smears

This brief morphological study of white cells red cells and other formed bodies in smears and in fresh blood should be supplemented by attempts by the students to see how many of the components already observed they can identify in fixed and stained sections of tissues

### SUMMARY

*Erythrocytes* are produced in the bone marrow When first formed by the division of the parent cells they are in the nucleated normoblastic stage of their life In the reticulocytic stage they possess a conspicuously stainable reticulum In the erythrocytic stage this reticulum has been lost and they serve, while dead in the blood stream as carriers of oxygen and carbon dioxide In this last stage they are elastic biconcave lens shaped bodies rich in hemoglobin characterized by great resistance to mechanical injury As living cells they could not stand repeated trauma so long *Platelets* are fragments of the cytoplasm of megakaryocytes of the bone marrow and possibly of other cells as well They are smaller than any blood cells consist of a hyaline looking ground substance in which granules are imbedded and they are not quite dead Platelets play an important part in blood coagulation *Chylomicrons* are droplets of fat encased in thin protein films *en route* from the area of intestinal absorption to the tissues *Other particles* are of several sorts but are not numerous

## CHAPTER III

### BONE MARROW

IN tracing the lives of blood cells it was necessary to include reference to their origin and fate and thus to consider what happens in some extravascular situations outside of the blood stream. We now pass to the principal site of production of the myeloid series (granular leucocytes and erythrocytes) in bone marrow and defer a further description of the lymphoid series (lymphocytes and monocytes) until the lymphatic system is taken up.

But development of the cells of both series has been reported far afield from the usual locations. The potentiality apparently exists wherever comparatively undifferentiated connective tissue cells exist. Formation of blood cells is designated hematopoiesis (*G harma*, blood + *poieo*, I make). When the myeloid series is produced outside of the bone marrow the phenomenon is known as *extramedullary myelopoiesis*.

**General Properties.**—The volume of all the bone marrows taken together is greater than one might expect. In adults it is about 5 per cent of the total body weight. Marrow is of 2 sorts: red, which is productive of blood cells, and yellow, which is fatty. A quantitative study of neutral fat, solids and water has been made by Huggins, McFadyen and Wieve (1940). In children bone marrow is nearly all of the red variety. In adults, whose bones are larger, not all of the available space within them is required to house red marrow. The balance is occupied by yellow marrow. This is an admirable arrangement. No lighter filling material could be found that increases when less red marrow is demanded and decreases, when more is required. The mechanism involved in this give and take is not known.

One factor may be local rate of oxidation. Fat is accumulated when it is not burned and disappears when it is burned. And fat is offered in chylomicron form to all vascularized tissues. The speed of chemical changes, including oxidation, is increased by rise in temperature. Huggins, Blocksom and Noonan (1936) have discovered that in the animals studied there is a temperature gradient extending from centrally placed to distally located marrow near the ends of the extremities, where it is 4 to 8° C. lower. Huggins and Blocksom (1936) have also observed that the red marrow decreases in relative amount and the yellow increases in passing from deep to peripheral tissues. They performed numerous experiments such as bending rats' tails and inserting them into abdominal cavities, thus increasing the temperature, and found that the red marrow was thereby increased in amount at the expense of the yellow. Evidently the tissue fluid environment of developing blood cells must be maintained at a high temperature just as that of the testicle must be held at a low temperature in order to produce sperms (p. 322).

Obtain a humerus or femur or other long bone, split it longitudinally and note the amount, color and consistency of the marrow. Take out small amounts of marrow and examine microscopically in a preliminary way. It is helpful for a few selected students to go to the clinic and watch the removal for diagnosis of bone marrow by sternal puncture. Two cc. can be secured easily by this technique which is sufficient for many smears (or impression preparations) if they are made by the instructors. One can be given to each student dried in air for staining by Wright's method and for examination; but if time permits it is better to study marrow cells fresh in physiological salt solution without any artificial coloring (Technique, p. 36).

**Identification of Cells**—Recognition of cells, which are sufficiently differentiated to exhibit specific properties is a simple matter when one begins with those already studied in the blood. Again the color blind students will be handicapped by difficulty in distinguishing hemoglobin but they will at least be able to make out the *erythrocytes* and *reticulocytes* already described. Great diversity in size will be met with (Fig. 28)



FIG. 28. Range of variation in size of bone marrow cells. Almost the whole figure is occupied by a megakaryocyte, the nucleus of which is marked by a circular clear space perhaps originally a fluid vacuole. On the right is a juvenile leucocyte and below and on the left a normoblast. From a smear of normal bone marrow obtained by sternal from a 49 year old female. Colored by Wright's stain and counterstained by Giemsa's.  $\times 1800$  (Lent by Dr. Carl A. Moore.)

**Normoblasts** (Figs. 23, 24, 28-30) are slightly larger or about the size of erythrocytes. Each possesses a small usually spherical deeply staining nucleus imbedded in cytoplasm charged with almost as much hemoglobin as an erythrocyte. The color of the cytoplasm is however slightly darker than that of an erythrocyte. The only other cells of the same size or smaller than normoblasts in the smears are

lymphocytes These, also, have spherical, deeply staining nuclei, but their cytoplasm is generally less in volume in relation to that of the nucleus and it does not contain hemoglobin Close examination may show that the nuclei of lymphocytes are colored slightly differently from those of normoblasts, being a little more purplish and less blue It is likely that there will be more normoblasts than such small lymphocytes; but this is not a good criterion for recognition because little islands of lymphocytes may occur especially in active red marrow of persons over forty years of age (Williams, R. J , 1939)

*Erythroblasts* (Fig 23) are distinctly larger than normoblasts Their nuclei are, as in the normoblasts, approximately spherical but they are much larger and stain

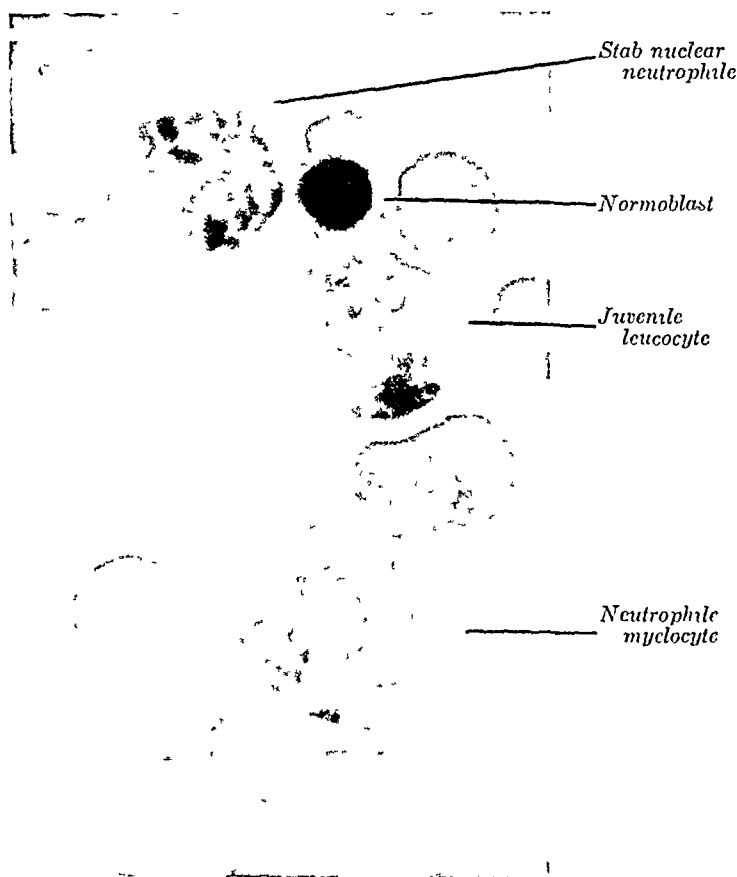


FIG 29 —Bone marrow of same specimen as figure 28

less intensely Their cytoplasm colors differently, because less hemoglobin is present and more basophilic material Those that differ least from the normoblasts, and in which there is considerable hemoglobin, are called "polychromatic" because their cytoplasm stains several colors varying from gray to lilac (G *poly*, many + *chroma*, color). The others, in which there is only a trace of hemoglobin, are termed "basophilic" because their cytoplasm takes the basic component of the mixed stain which in this case is blue They are less differentiated than the polychromatic erythroblasts

*Neutrophile myelocytes* (Figs 23, 29-31) are larger than the corresponding leucocytes contain similar granules and larger, less lobated and less deeply staining nuclei



*Eosinophilic myelocytes* (Fig 23) similarly differ from the corresponding leucocytes being larger and possessed of a less lobated and less intensely staining nuclei. They are less numerous than the neutrophilic ones.

*Basophilic myelocytes* (Fig 23) can, in the same way, be distinguished from basophilic leucocytes. They are the least numerous of the three.

But not all myelocytes differ from their leucocytes in the same degree. On the contrary, when many are examined, a step like series is found passing down from the leucocytes to primitive cells devoid of specific granules in which

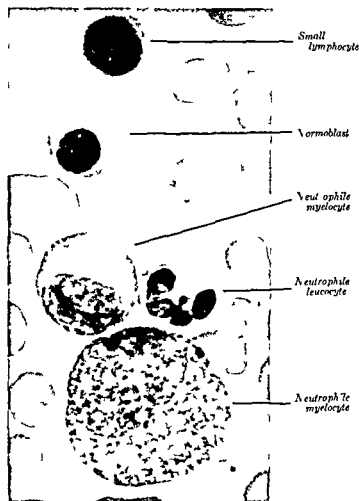


FIG. 30.—Bone marrow of same specimen as figure 28

- 1 Cell size increases
- 2 Nucleocytoplasmic ratio increases
- 3 Nucleus becomes more spherical
- 4 Basophilia of cytoplasm increases and
- 5 Specific granules decrease to the point of being just detectable

Some hematologists would split hairs and divide the myelocytes of the 3 types into groups depending upon degree of differentiation. Students need not at this stage be expected to do so. Neither should they have to flounder about in attempts to distinguish among the *primitive cells* which contain no trace of hemoglobin or of specific granules, those which give rise to erythroblasts and myelocytes respectively.

Precursors of myelocytes are commonly called *myeloblasts* (Fig. 23). Volumes have been written about them. An authoritative statement is that of Downey (1938). It is fortunately supplemented by excellent colored illustrations. These cells are of variable size, but their nuclei are always large in proportion to their cytoplasm. In stained smears their nuclear membranes are extremely thin and indistinct. The chromatin is finely divided and stains faintly. Some of it may be condensed about the nucleoli of which several are usually seen.

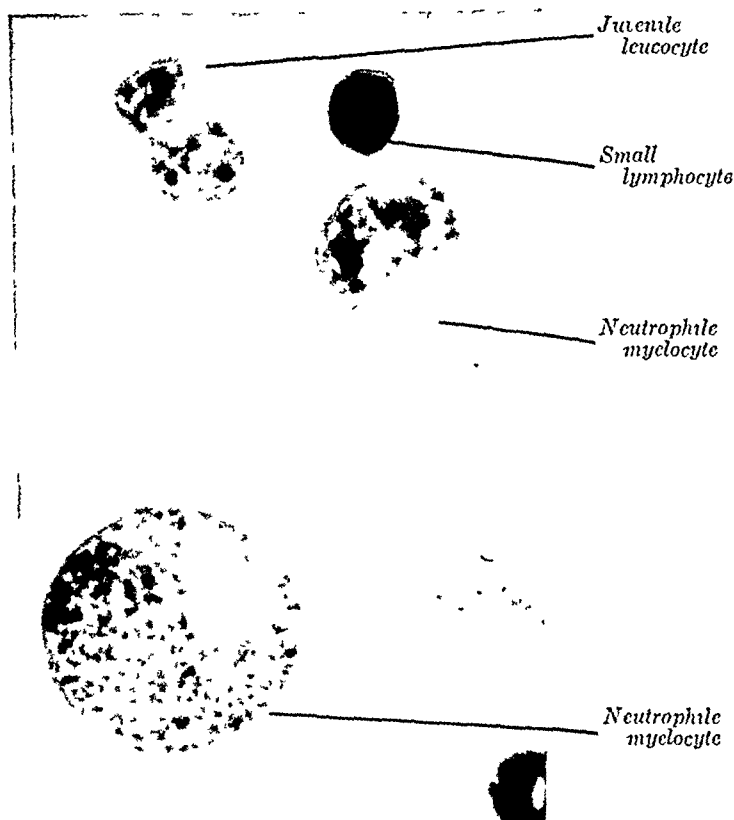


FIG. 31 — Bone marrow of same specimen as figure 28.

No difficulty will be experienced in the identification of *megakaryocytes* (Fig. 27). This term, being translated, means large nucleus cells and they are truly gigantic compared with all other cells present except the polykaryocytes. Their cytoplasm should be examined for signs of the breaking off of platelets.

*Polykaryocytes* are giant cells possessed of many nuclei of fairly uniform size. They do not participate in blood cell formation (see p. 296).

Since the smears were purposely made from red bone marrow, it is improbable that many fat cells were included. But marrow is more than blood. Some capillary endothelial cells will be present, as well as reticular cells, associated with fibers here and elsewhere, which some claim in this location to be the ultimate source of the granular leucocytes. In making the smear everything is mixed up together and many cells are seriously injured. It is not uncommon to find nuclei here and there from which the cytoplasm has been torn and which themselves may be drawn out in strands.

**Cell Lives.**—So much for the easily observable properties of marrow cells concerned in blood formation. What is to be our mental picture of their individual

*Eosinophilic myelocytes* (Fig 23) similarly differ from the corresponding leucocytes being larger and possessed of a less lobated and less intensely staining nuclei. They are less numerous than the neutrophilic ones.

*Basophilic myelocytes* (Fig 23) can in the same way be distinguished from basophilic leucocytes. They are the least numerous of the three.

But not all myelocytes differ from their leucocytes in the same degree. On the contrary, when many are examined a step-like series is found passing down from the leucocytes to primitive cells devoid of specific granules in which

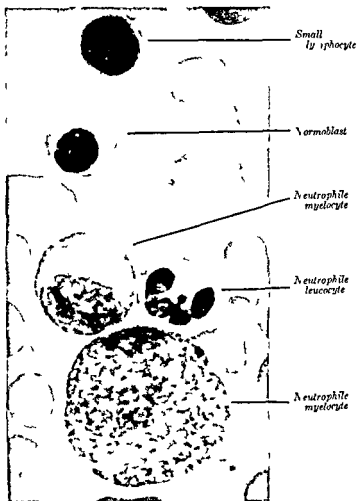


FIG. 30 — Bone marrow of same specimen as figure 28

- 1 Cell size increases
- 2 Nucleocytoplasmic ratio increases
- 3 Nucleus becomes more spherical
- 4 Basophilia of cytoplasm increases and
- 5 Specific granules decrease to the point of being just detectable

Some hematologists would split hairs and divide the myelocytes of the 3 types into groups depending upon degree of differentiation. Students need not at this stage be expected to do so. Neither should they have to flounder about in attempts to distinguish among the *primitive cells* which contain no trace of hemoglobin or of specific granules those which give rise to erythroblasts and myelocytes respectively.

Precursors of myelocytes are commonly called *myeloblasts* (Fig. 23). Volumes have been written about them. An authoritative statement is that of Downey (1938). It is fortunately supplemented by excellent colored illustrations. These cells are of variable size, but their nuclei are always large in proportion to their cytoplasm. In stained smears their nuclear membranes are extremely thin and indistinct. The chromatin is finely divided and stains faintly. Some of it may be condensed about the nucleoli of which several are usually seen.

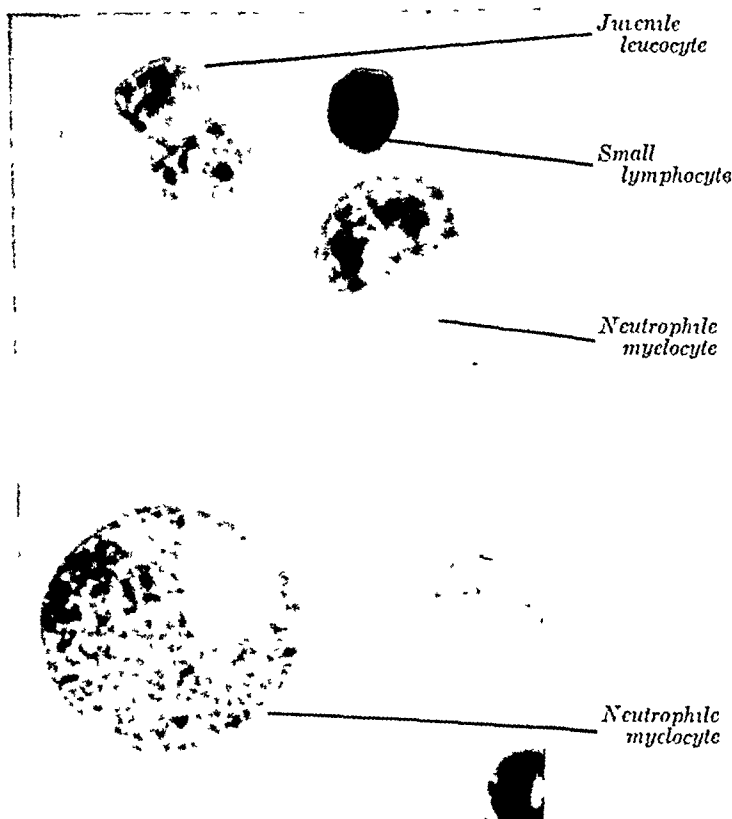


FIG. 31 —Bone marrow of same specimen as figure 28

No difficulty will be experienced in the identification of *megakaryocytes* (Fig. 27). This term, being translated, means large nucleus cells and they are truly gigantic compared with all other cells present except the polykaryocytes. Their cytoplasm should be examined for signs of the breaking off of platelets.

*Polykaryocytes* are giant cells possessed of many nuclei of fairly uniform size. They do not participate in blood cell formation (see p. 296).

Since the smears were purposely made from red bone marrow, it is improbable that many fat cells were included. But marrow is more than blood. Some capillary endothelial cells will be present, as well as reticular cells, associated with fibers here and elsewhere, which some claim in this location to be the ultimate source of the granular leucocytes. In making the smear everything is mixed up together and many cells are seriously injured. It is not uncommon to find nuclei here and there from which the cytoplasm has been torn and which themselves may be drawn out in strands.

**Cell Lives** —So much for the easily observable properties of marrow cells concerned in blood formation. What is to be our mental picture of their individual

lives? Progression is orderly, generation after generation with daughter cells resulting from mitosis beginning at about the stage of differentiation achieved by their parents and carrying it a little farther by developing a higher concentration of hemoglobin more specific granules or some other specialized feature. This advance they in turn pass on to their descendants when their own individual lives are terminated by mitosis. They are what are called *differentiating intermitotics*. Specialization finally reaches its peak of usefulness in which postmitotic cells are made ready to serve. These are removed from the scene without offspring. Obviously if the supply of basic undifferentiated cells at the root of any series were to become exhausted that series would in a number of generations still unknown differentiate itself out of existence. These primitive root cells are designated *vegetative intermitotics*. When they multiply some of their daughter cells continue as vegetative intermitotics, while others placed a little differently perhaps start on the path of differentiation. Only the continued production of such vegetative intermitotics makes possible the orderly replacement of old cells by new ones in many parts of the body. It is particularly necessary for blood cells because of the hazardous nature of their occupation. Though the finished leucocytes and reds are incapable of division and cannot embark upon careers of malignancy the intermitotic cells are able to do so if adequately deranged. It is to the antecedent cells of the leucocytes and also of the lymphocytes that we must look in efforts to explain the myelogenous and lymphatic leukemias.

A simple calculation shows that in the circulation the numerical ratio of granular leucocytes to erythrocytes is about 1:800. This does not mean that for every leucocyte formed in the bone marrow 800 erythrocytes are developed, because the latter once supplied remain longer in the circulation. They do not persist however anything like 800 times as long. Consequently one would expect more marrow cells to be involved in the production of erythrocytes than of leucocytes. But the contrary is true. When only the differentiating intermitotics marked by the presence of some hemoglobin or of some specific granules are listed the ratio of leucocyte to erythrocyte producers is approximately 3:1. If, in the two groups the length of intermitotic life were the same one would anticipate in view of this 3:1 ratio 75 mitoses among the whites to 25 among the reds. But Japá (1942) found that this is not the case. His counts show approximately 45 whites in mitosis and 75 reds in each 100. Therefore it appears that the reds are multiplying more rapidly than the whites and that their intermitotic lives are shorter.

**Numerical Increase**—To read the messages transmitted from blood to bone marrow demanding more cells of either sort to keep the number in the circulation up to normal is beyond us. Maintenance of cell number is not peculiar to the blood. It is a property of all replicable tissues. Thus along the respiratory, digestive and urinogenital tracts the number of cells making up the epithelial linings is held fairly constant. Loss of an unusual number from the surface is made good by the production of a larger crop of new ones by the deeply situated vegetative intermitotics eventually to take their places. Both in these situations and in the bone marrow rate of multiplication probably increases in response to some change in the immediate environment. Since vegetative intermitotics and differentiating neutrophilic, eosinophilic and basophilic intermitotics are closely packed together it is unlikely that the change embraces only the cells productive of granular leucocytes of a single category. Yet the neutrophiles may increase in the circulation independently of the eosinophiles and *vice versa*. Responsiveness or lack of it to

an environmental change may make all the difference. Obviously, however, increase in number of any particular sort of cell in the peripheral blood stream may be occasioned by a temporary increase in discharge while some of them are immature from bone marrow, the rate of production in the marrow remaining about the same. Another possibility is that, though the total number in the circulation may not be increased, the number in the peripheral blood is increased in consequence of a redistribution whereby they are washed out from deeper channels in which they may be normally more numerous. The sudden increase in eosinophiles within two and one-quarter minutes (p. 28) may be a case in point.

**Differential Counts.**—In practice it may be worth while to try to discover in the bone marrow the reason for unusual conditions observed in the blood. Search then is made for abnormal looking cells in the bone marrow and for disturbances in the relative number of cells of different types. The making of differential counts of marrow cells is a comparatively new development in hematology. But there is a good deal of confusion because some of the kinds recognized are not sharp and distinct but grade one into the other. A useful summary of the results of counts of normal marrow cells made by many investigators is provided by Plum (1941).

### SUMMARY

The myeloid series consists of granular leucocytes, erythrocytes and the cells leading up to them which are formed in the bone marrow. Bone marrow is of 2 kinds, red and yellow. The first is a blood producer and the second is a fatty tissue that occupies the extra space within the bones. Red marrow cannot expand quickly like a gland. It can only extend slowly at the expense of yellow marrow. When it decreases in volume yellow marrow takes its place. The best clues to identification of the differentiating cells are hemoglobin, neutrophilic, eosinophilic and basophilic granules.

*Normoblasts* are about the same size as erythrocytes. Each contains a small, deeply staining nucleus and much hemoglobin. *Erythroblasts* are larger, have less intensely staining nuclei and less cytoplasm. *Myelocytes* of the 3 varieties are larger than the corresponding leucocytes and have less deeply staining and less rounded nuclei. Their specific granules, like the hemoglobin of the erythroblasts, may be present in amounts barely discernible. Each exhibits a large nucleocytoplasmic ratio and slightly basophilic cytoplasm. *Primitive cells* which have no hemoglobin and no specific granules are difficult to identify except by these negative characteristics. It is the intermitotic cells that are subject to the cancerous transformation. *Megakaryocytes* (large nuclei cells) recognizable by their size are apparently the source of platelets. *Polykaryocytes* (many nuclei cells) take no definite part in blood formation.

## CHAPTER IV

### BLOOD VESSELS

THUS far the most easily examined of cells have been described—those of the blood. Brief mention has been made of the tissues in which blood cells originate and to which some later may return for otherwise the account would be artificial limited only to segments of their lives. It is necessary now more formally to introduce the tissues.

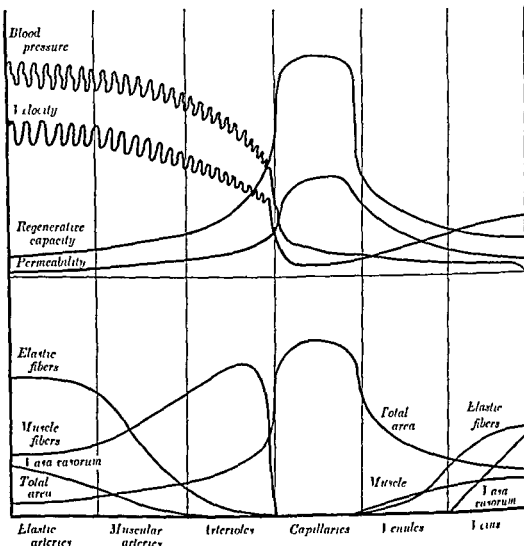


FIG. 32.—Graph illustrating some of the changes in structure (below) and function (above) of vessels in the direction of blood flow from ventricle to auricle.

Blood is sometimes spoken of as a circulating tissue. This is not good form and may be misleading. The word tissue is immediately derived from the French *trava* textile fabric and ultimately from the Latin *texere* to weave. Used histologically, tissue denotes a group of cells with intervening tissue fluid and any

products they may form, the whole constituting a definite structure. The connective tissues are of particular interest to us now. They are made up of cells, tissue fluid and fibers produced under the influence of certain of the cells (fibroblasts and/or reticular cells). The fibers are arranged singly and in bundles and wind in and out between the cells. In "loose" connective tissue there is a good deal of fluid, in fibrous connective tissue the fibers predominate, in fatty tissue, the cells become greatly distended with fat and the fibers are inconspicuous; while in bone and cartilage the tissue fluid becomes solid. Knowledge concerning the connective tissues should be cumulative as each is examined in its own environment.

The blood in its journey from the heart to the tissues and back again passes through vessels of 6 principal kinds which gradually merge one into the other. Some features in their changing form and function are indicated graphically in figure 32.

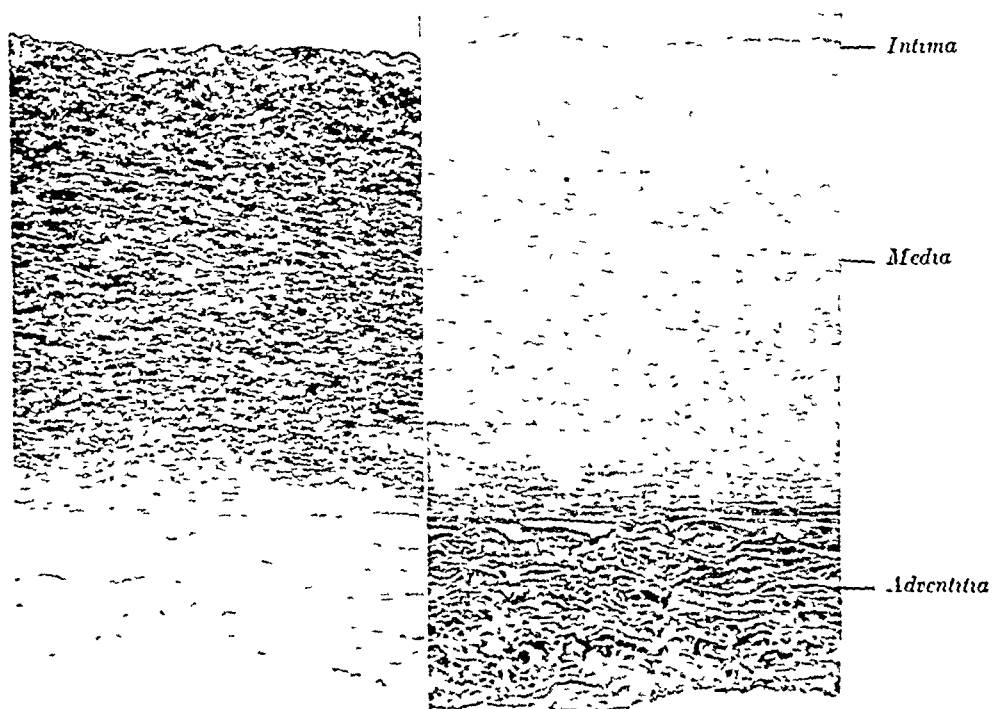


FIG 33 —Aorta, nineteen year old female. Motor accident, autopsy twelve hours after death. Formalin-Zenker fixation.  $\times 64$ . Section on left stained for elastic fibers which are abundant in intima and media. Section on right stained by Van Gieson's method for collagenic fibers which are most numerous in the adventitia. (Dept. Pathology, Washington University, No. 10241, tissue given by Dr. John A. Saxton, Jr.)

**Elastic Arteries** —Blood is forced by ventricular contractions at high pressure into the aorta and pulmonary artery. These two, together with the innominate, subclavians and proximal parts of the common carotids are known as elastic arteries. The full force of the heart's beat is not immediately transmitted to the smaller, more distant arteries as would be the case were the elastic arteries rigid tubes. Instead they expand, part of the force is converted into pressure which is released slowly while the ventricles are at rest (diastole). During this period blood is forced onward by the recoil of the stretched elastic tissue in their walls. Elastic arteries therefore serve as shock absorbers converting a pressure, which otherwise would be



intermittent, into one which though still varying regularly in intensity is nevertheless continuous. A good description of mechanical factors in the circulation is given by Price and associates (1942)

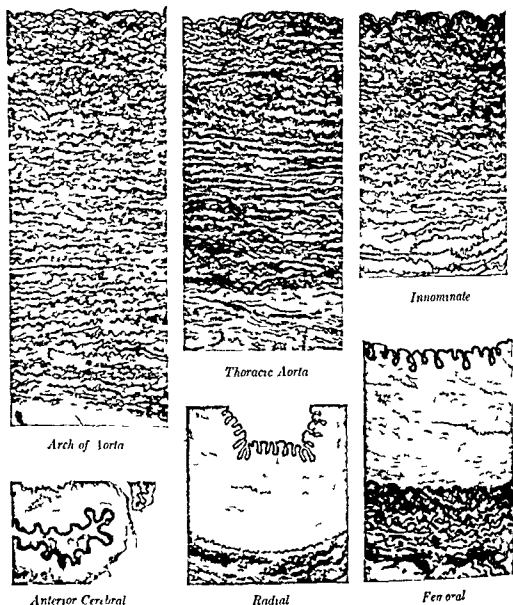


FIG 34—Photomicrographs of walls of elastic and muscular arteries of a large male *Macacus rhesus* monkey about fifteen years old. The elastic tissue was colored dark purple in the sections by staining with resorcin fuchsin but, in the upper 3 figures not all of the adventitia is included. Note comparative thickness, arrangement and amount of elastic tissue.  $\times 140$

Figure 33 shows neighboring sections of an aorta stained for elastic fibers (left) and collagenic fibers (right). Evidently elastic fibers are concentrated in the thin innermost layer (intima) and in the thick middle layer (media) whereas the collagenic fibers noted for their tensile strength are most abundant in the outer layer (adventitia). This latter is applied to the other and is in a sense foreign (*adventicius* foreign). Hence the name

Winternitz and his associates (1938) have observed that human aortas with adventitia intact can be distended only to a sharply limited extent but that, after incision of the adventitia, dilatation with rupture of the remaining elements is easily brought about. In other experiments they subjected extirpated dogs' carotids to an internal pressure of 2500 mm of mercury with slight or no increase in diameter over that resulting from relatively low pressure of 250 to 300 mm. Incision of adventitia was followed by rupture even at low pressures.

In an automobile tire, the inner tube (intima + media) is restrained from undue expansion by the tire casing (adventitia). The casing is not elastic like the inner tube but it is stronger because woven in its fabric is material of great tensile strength as the adventitia has collagenic fibers. One wonders whether, in the dilatations occasionally encountered in elastic arteries, ageing or failure of collagenic fibers of the adventitia, and also perhaps of the media, may not be a factor worthy of consideration as well as the change in the elastic fibers so often mentioned.

Figure 34 illustrates the fact that precise limitation between the layers is not so easily made in elastic arteries as in muscular ones (anterior cerebral, radial, femoral). This is because the internal and external elastic membranes do not stand out so sharply in the former owing to the large proportion of elastic tissue in the media.

The *intima* of the aorta consists of the lining endothelium, plus a thin layer of subendothelial connective tissue in which there may be a few muscle fibers, and the internal elastic membrane. The *media* is external to this membrane and includes the external elastic membrane. In addition to the elastic fibers, it contains some collagenic fibers and muscle as well as tiny blood vessels to keep the muscle alive and nerve fibers to activate it, and, of course, fibroblasts, since new fibers are occasionally formed. The *adventitia* grades off into the surrounding loose connective tissue. Through it vessels and nerves enter the media. No concept of the architecture of the wall of an elastic artery is at all adequate if obtained only from the study of sections.

Obtain a fresh human thoracic aorta from an autopsy. Open it longitudinally and demonstrate features of the smooth inner endothelial surface and of the outer adventitial surface. Remove 2 pieces. Place 1 in glycerin and the other in 10 per cent formalin for twenty-four hours. Divide the rest of the aorta in segments, about 3 cm long, and give to selected students for dissection (Technique, p. 24).

Pin the segment down and examine microscopically smears of scrapings of its internal surface for endothelial cells. Turn over specimen, cut away small pieces of adventitia, stretch out on slides and examine unstained for tiny blood vessels, nerve fibers, elastic and collagenic fibers and the fibroblasts that are concerned with their formation. The elastic fibers are few in number, thin and fairly highly refractile, while the collagenic fibers are very numerous, thicker, tend to split and swell when a little 5 per cent aq. acetic acid is added (see p. 272).

Separate adventitia from media. Note first its great tensile strength due to the abundance of collagenic fibers and when observed as a sheet of tissue at low magnification, the large number of small blood vessels in it. These are the *vasa vasorum*, the vessels of the vessels, on which the vitality of the aorta chiefly depends.

Remove small pieces of media. Note that it is yellower than the adventitia owing to the abundance of yellow elastic fibers. Attempt to separate the fibers on a slide, examine microscopically and identify elastic fibers, collagenic fibers and smooth muscle fibers (not present in adventitia).

After twenty-four hours examine the specimens treated with glycerin and formalin. The first is rendered quite translucent and close study shows many details not visible in the formalin control.

**Muscular Arteries** — Blood gushes into them at a slightly reduced pressure and at a speed which is also reduced because the combined cross sectional area of a great many muscular arteries taken together is greater than that of a few elastic vessels. Muscular arteries are also termed distributing arteries since they conduct the blood often over long distances to where it is needed. The muscle in their walls is often arranged in remarkable spirals (Fig. 36) as described in a fine piece of work by Strong (1938). Some consequences of the high proportion of muscle are

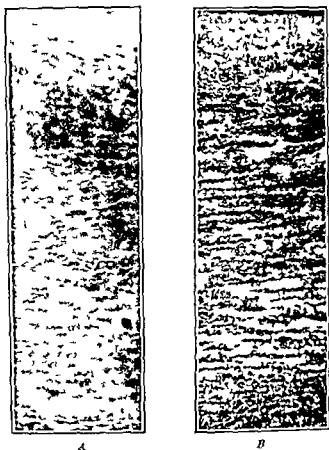


FIG. 3. — Preparations showing calcium deposition in the media of an elastic artery. *A*, Aorta (executed negro aged thirty five years) fixed in Zenker's fluid and stained with hematoxylin and eosin. Coloration with hematoxylin is unusually intense owing to calcium accumulation.  $\times 90$ . *B* Same, fixed in formalin alcohol incinerated and viewed in the dark field.  $\times 90$ . (Photomicrographs by Gordon H. Scott.)

(1) In the muscular arteries (Fig. 34) the internal elastic membrane is more folded than in the elastic ones. Galloway (1936) has made an investigation of fold formation in these arteries which are of the muscular variety. He discovered that it is greatly increased by agonist contraction of the smooth muscle at the time of death or when the piece is excised. Figure 37 shows the folds usually seen (left) and the smooth internal surface of a similar artery relaxed with potassium sulphocyanide and distended by systolic pressure (right). Decrease in length is also a factor to be considered in fold formation. Hesse (1926) measured the arterial trunk *in situ* from the beginning of the subclavian to the distal end of radial. She observed that after removal in young persons the decrease amounted to 40 per cent. This is commented on by Bell (1943) whose account will be found useful.

Extreme cases of the same phenomena are described in the literature under the heading of "traumatic arterial spasm." The walls of muscular arteries can contract so strongly that pulsation is not felt distally, or, in case the artery is cut, little if any blood escapes (see Arey, 1936, and Cohen, 1940-41). This feat of closure could not be performed by an elastic artery.



FIG 36 —Spiral strand of muscle obtained by stretching the macerated media of a branch of a cerebral artery of an adult.  $\times 8$  (Strong, courtesy of Anat Rec)

(2) Muscular arteries are also more vital in their ability to rise to the occasion and deliver the goods on demand (Fig 38). Elastic arteries are usually the only effective means of supply to large areas. They are not paralleled by others of the

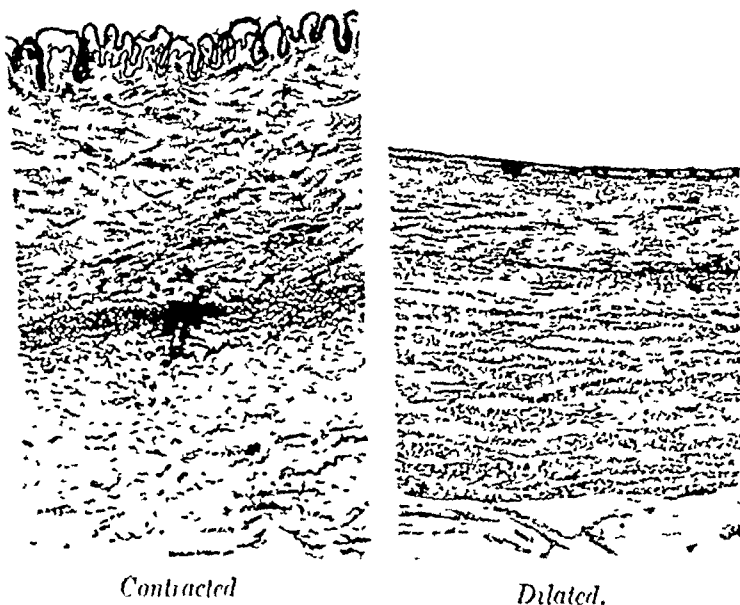


FIG 37 —Effect on structure of arterial wall of contraction on removal from body and dilation while carrying blood stream (Redrawn from Galloway, courtesy of Am. J Path)

same sort. Not so the muscular arteries which are often so arranged that, when one is blocked, blood can be rerouted *via* a collateral. In such cases the muscular arteries, called into heavy duty, enlarge many times. An outstanding example is

the uterine artery which grows with the need for more blood as pregnancy advances. After pregnancy it decreases in size adjusting itself to the volume of blood needed. This would be an impossible task for an elastic artery held as it is within bounds by a strong restraining adventitia.



16 days



48 days Enlarged arteries near infected area



60 days Reduction in area of infection and in extent of chronically enlarged vessels



60 days Recovery infection has disappeared vessels again normal

FIG. 38—Shows reaction to infection of blood vessels in a chamber at the times indicated inserted into a rabbit's ear  $\times$  about 82. (Modified from Clark, E. R. and E. L. courtesy of Am. J. Anat.)

(3) It is moreover the muscular arteries that can best recover from wounds. Figure 39 illustrates stages in the joining of an artery at the point where it has been severed.

(4) Since they have relatively so much more muscle, muscular arteries age differently from elastic ones. The muscle atrophies as in most other situations throughout the body. The length of service of elastic arteries depends chiefly on

the inherited quality of the elastic colloid and on the changing character of the tissue fluid environment in which it ages (Fig 40) On the ageing of elastic tissue see Hass (1942).

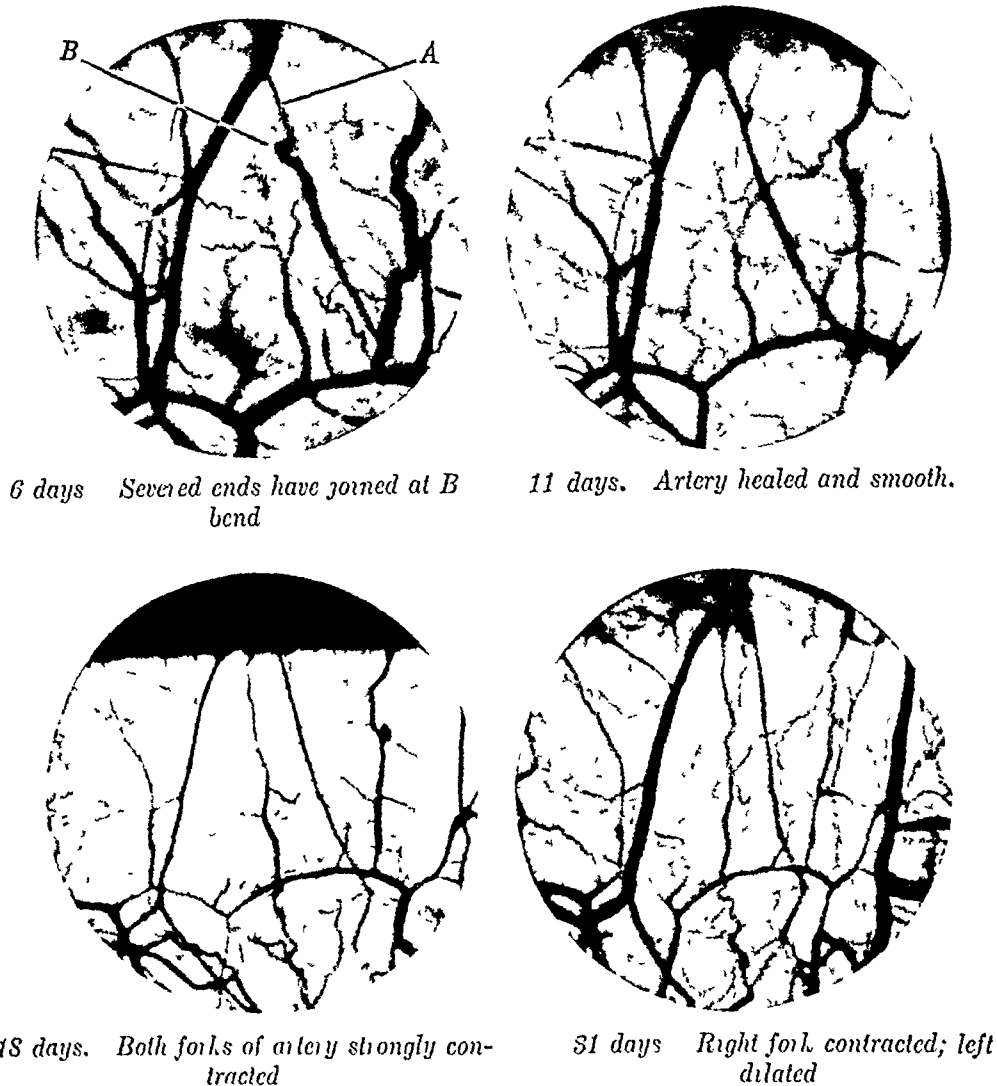


FIG 39 —Shows healing and complete recovery of an artery severed when a chamber was inserted in a rabbit's ear. The artery is the right fork of a vessel appearing to enter the chamber from above and is marked A. The time after cutting it is given and the location of the cut is marked B.  $\times 65$  (From Clark, E. R. and E. L., courtesy of Am. J. Anat.)

Many other differential features might be mentioned but the dictates of available space in this book forbid. Sufficient has been said to indicate that the distinction in form and function between elastic and muscular arteries is definite, but the point where a particular vessel changes from elastic to muscular, as one passes away from the heart, may not be clearly defined. Sometimes the transition is marked by a somewhat irregular increase in muscle of the media, and by other properties, in which case the transitional portion is said to be of the mixed type.

**Arterioles.**—These are "little arteries" and the blood enters them at still further reduced pressure and more slowly (refer back to Fig 32). Their diameter in sections of fixed tissues is given by various authors as from  $25$  to  $300\mu$ . But this upper limit is probably too high. It is well to restrict the term to small arteries which are invisible

to the naked eye which would place their maximum diameter at about  $150\mu$ . Three typical arterioles are shown in figure 41. Distinctive properties relate more to the character of the wall than to size. The ratio of thickness of wall to diameter of lumen is about 1:2. There is relatively more muscle than in any other segment of the circulation. In addition to the large complement of medial muscle they possess a little collagenic and elastic tissue chiefly in the adventitia. The media often undergoes hypertrophy and it becomes necessary to gauge the degree of change from normal. In so doing the figures supplied by Kernohan, Anderson and Keith (1939) and by Biker (1941) will be helpful. Other useful illustrations are provided by Benninghoff (1930) who gives the classification of arteries prevailing in Europe. With decreasing size the term arteriole is justified as long as the little artery exhibits a complete layer of typical, fusiform smooth muscle cells (Fig. 42).

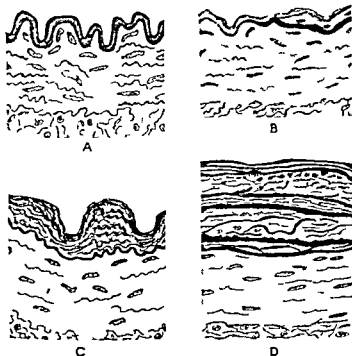


FIG. 40.—Sections of artery walls from individuals at different ages. All are stained with Hart's modification of Weigert's elastic tissue stain. A, Vertebral artery from an infant of fifteen days. No splitting of the lamina elastica interna is seen.  $\times 350$ . B, Vertebral artery from a child aged thirteen years; the lamina elastica interna is split into two layers at one point.  $\times 200$ . C, Median cerebral artery from an adult aged thirty-two years; there is marked multiplication of the lamina elastica interna.  $\times 200$ . D, Posterior cerebral artery from a man aged fifty years showing the highest grade of splitting up of the elastic lamina.  $\times 230$ . (Redrawn and modified from Hackel, *Virchow's Arch.*)

Arterioles occupy a strategic position in the circulation between the muscular arteries on the one hand and the capillary bed on the other. Their function is properly to deliver blood to the capillaries. This is supplied in continuous streams for the oscillations in pressure and speed consequent upon the ventricular contractions have been ironed out. Because of their highly muscular walls their girth is very directly and completely under nervous control. By constriction all blood flowing through them can be shut off. By relaxation their lumina dilate under the head of pressure in the muscular arteries and more blood is permitted to pass through. Exactly how vasodilator nerve fibers act is unknown. Presumably a

## ARTERIOLES

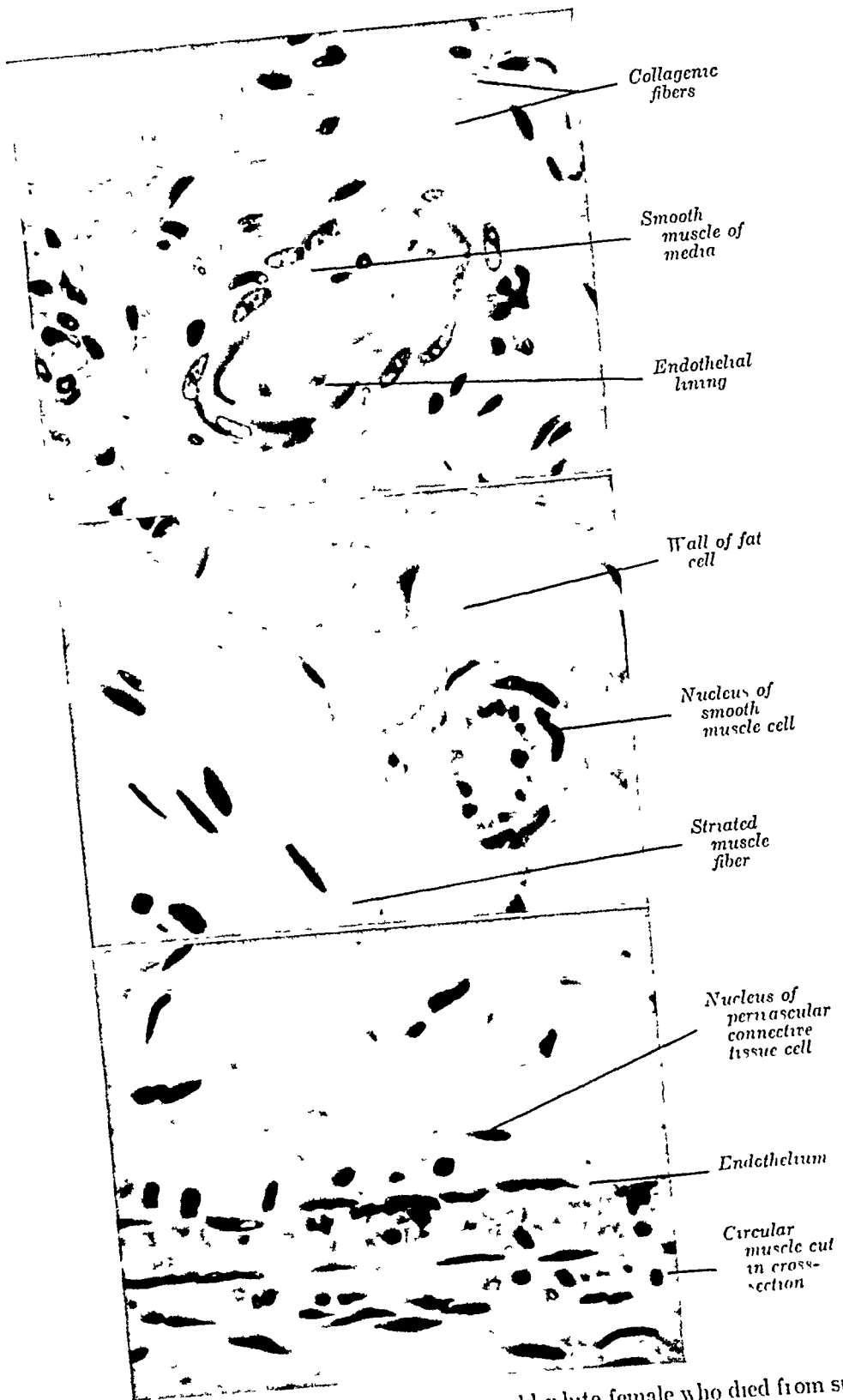


FIG. 11.-- Arterioles in tongue of a thirty-two year old white female who died from subdural hemorrhage. Fixation in Kaiserling's solution-1 and 3, H & E  $\times 540$  (Barnes Hospital, No. 10039B, tissue given by Dr. R. E. Stowell).



substance is released in the muscle which decreases its tonus (resistance offered to extension) and leads to an unusual degree of relaxation. Such vasodilation can happen within a few seconds as in blushing. Constriction, when widespread can lead to increased arterial blood pressure by increasing peripheral resistance to the flow of blood pumped into the elastic arteries by the heart. Similarly sudden wholesale relaxation can produce decrease in blood pressure and shock by decreasing peripheral resistance so that much blood spills into the capillary bed whence it very quickly passes into the veins which can accommodate a lot of it.

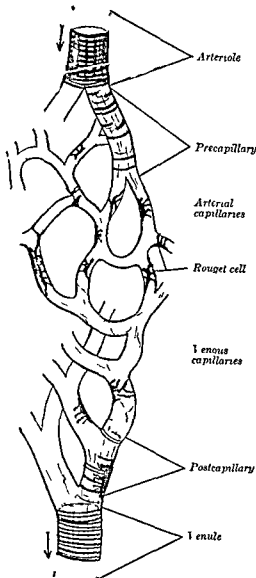


FIG. 42—Diagram of transition from an arteriole to a venule

An excellent demonstration of arterioles in action is given by a motion picture film entitled "The Control of Small Blood Vessels" by Drs. George P. Fulton and Brenton R. Lutz of the Dept. of Biology, Boston University.

**Capillaries**—Blood is pushed into the capillaries at such low pressure that the walls of its myriad streams no longer require organized mechanical support and consist only of a single layer of flattened endothelial cells. The only reinforcement provided consists of (1) a few scattered smooth muscle cells which partly encircle the precapillaries and postcapillaries (Fig. 42) and (2) of occasional 'pericytes' whose many processes may be wrapped about the capillaries (Fig. 43). Among the latter are some contractile elements known as Rouget cells. Great stretches of the capillary bed are without cells of either sort. Krogh has calculated that the surface of exchange of all capillaries between blood and surrounding tissue fluid is about 6300 sq. meters. Since the combined cross-sectional area of these countless capillaries is reliably stated to be approximately 800 times the cross-

sectional area of the blood stream at the aortic level it follows that the rate of blood flow is slowed down so that time is given for the exchange. But the whole surface is seldom used at any one time. During rest of a tissue fewer capillaries are carrying blood than at the height of functional activity as Krogh showed for muscle. The volume of blood circulating in any capillary bed depends for the most part upon the amount entering by the arterioles and that leaving by the venules. The influence of active changes in girth of the capillaries themselves is uncertain. By the delicate technique of micromanipulation Zweifach (1934-1936) has observed

that the endothelial walls respond by slow contraction to direct mechanical stimulation and by dilation from the contracted state when a widespread and gentle mechanical stimulus is applied throughout their length. If possible his moving picture film, "Micromanipulation Studies on Blood Capillaries," should be demonstrated. In a later paper (1939) he expressed the opinion that normally "the changes in capillary diameter which occur are slow and passive"

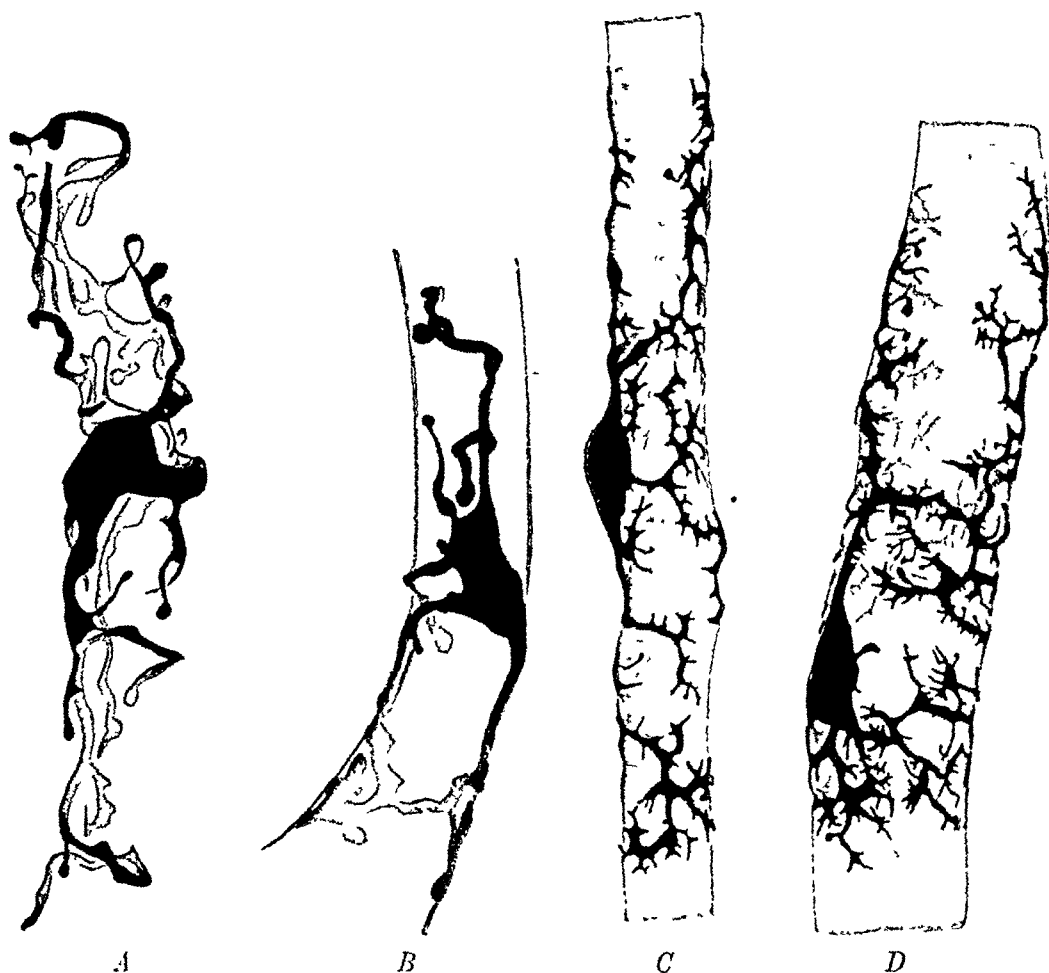


FIG 43 — A and B Post capillary pericytes from 15 microns thick venula recta of a forty-three year old man C and D same from 10 microns and 16.7 microns thick veins of the heart of a young cat  $\times 1500$  (Zimmermann, Zeit f. Anat, 1923; courtesy of Julius Springer)

Nerve fibers are often closely associated with capillaries. Stohr's (1926) illustrations of fibers said to end (or begin) within the substance of endothelial cells will repay study. Employing a new technique which permits better visualization of capillaries in rabbit ear chambers Sanders, Ebert and Florey (1940) have observed capillary contraction to follow sympathetic stimulation. The lumina are occluded by a swelling of the endothelial cells. Any increase in thickness of the wall presumably decreases permeability just as dilatation and thinning, whether from increased intracapillary pressure or from relaxation of the wall, increases permeability. Leakage along the lines of contact between endothelial cells is apparently greater than that through the cell bodies (Chambers and Zweifach, 1940). Evidence is accumulating that adequate amounts of vitamin P are essential for the

maintenance of normal capillary permeability (Rapaport and Klein 1941) Capillary walls are the most vital segment of the circulation because they are made up solely of living cells. New capillaries can develop as long as the body lives. Consult review of literature by Hertzman (1942)

**Venules** —As the blood still backed by pressure from the heart passes into the venules the speed of flow increases as the combined cross sectional area decreases (Fig. 32). With increase in size the venules acquire more muscle and collagenic and elastic fibers but their walls are never as thick as those of the corresponding arterioles (Fig. 44)



FIG. 44 — Arteriole and venule from same specimen as figure 41

Leucocytes can move out when needed. To this end the lining endothelium undergoes changes in physical consistency or stickiness which have been directly observed in living animals by the Clarks (1936). They recognize six phases represented schematically in figure 45. In the first the leucocytes pass by freely in the blood stream. In the second and third they stick to the wall but do not penetrate through it. In the fourth they emigrate and in the fifth red cells escape. Even at this point recovery is possible by reversion to the preceding phases as indicated by the arrows pointing upward. In the sixth phase there is disintegration of the endothelium and recovery is no longer possible. Penetration of leucocytes through capillary walls is also a simple matter.

Rous and his collaborators have described a gradient in permeability which increases progressively from arterial to venous capillary but is greatest in the venules at any rate in the skin of the mice experimented upon (Smith and Rous 1931). Their measure of permeability was the escape of dyes of different diffusibility. Figure 46 shows how a few minutes after intravenous injection the dye Chicago blue colors the tissue about the venules but not that supplied by capillaries. The authors contend that such experiments with dyes indicate that a similar gradient in permeability obtains for other kinds of substances but do not supply proof.

Where venules (little veins) end and the veins proper begin is as difficult to define as the point of transition between muscular arteries and arterioles. When a venous vessel attains a size at which it is visible to the naked eye *in vivo* it should be called a vein.

**Veins.**—In general in the transition from venules to veins, as they approach the right auricle, three major changes take place (1) The combined cross sectional area decreases so that speed of blood flow increases (2) the blood pressure decreases, partly as distance from the ventricular contractions increases (3) The vasa vasorum

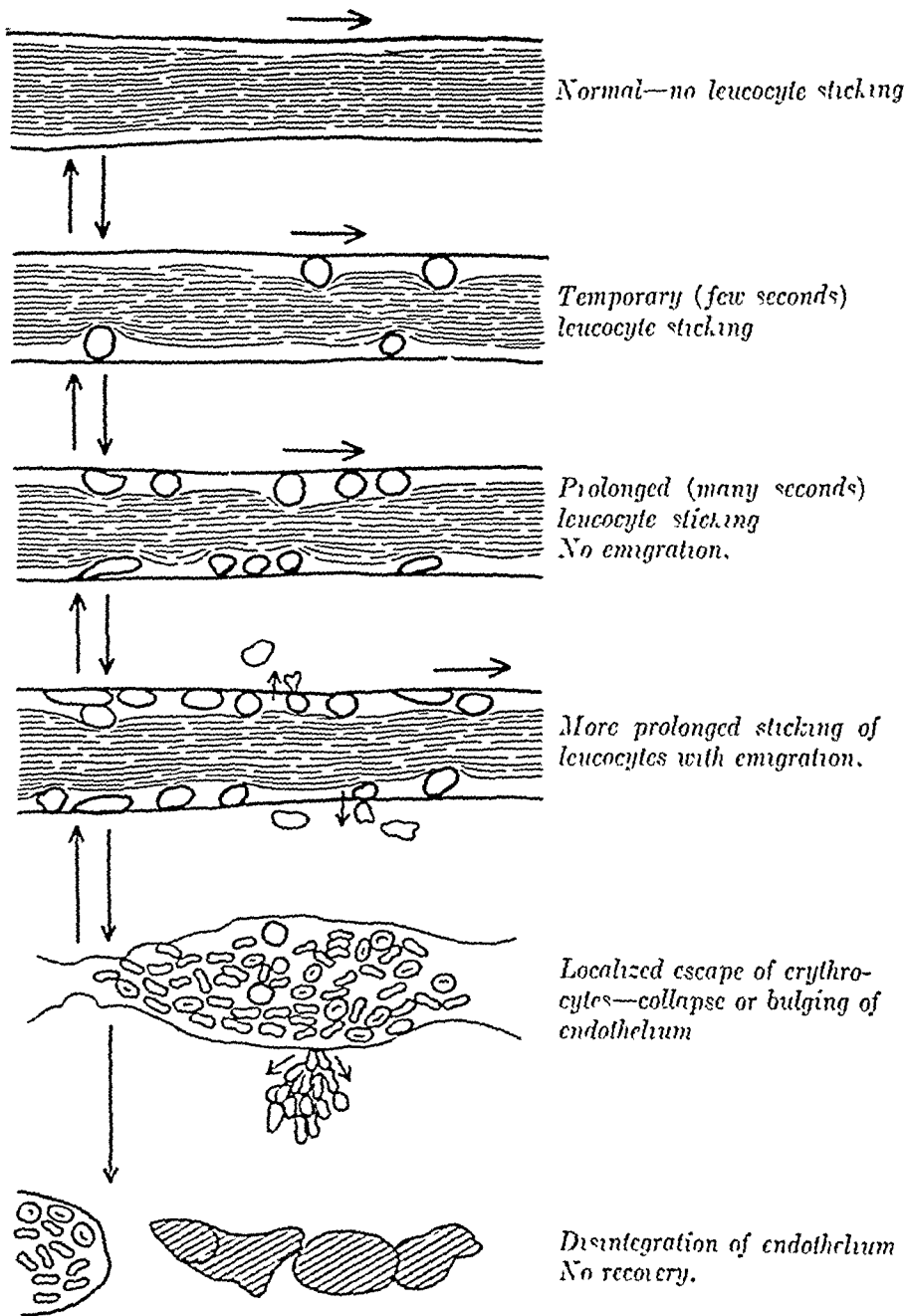


FIG 45.—Diagrammatic representation of changes in consistency of vascular endothelium (Clark, E R and E L, courtesy of Am J Anat)

increase in number and size with the thickness of the wall to be nourished from the adventitial arterioles because only venous blood is carried in the lumina (Examine in this connection Short's [1940] account of vasa vasorum of the femoral vein)

While the arterial blood flowing to all the tissues has enjoyed a common experience in the lungs, the venous blood comes from capillary beds of great variety and

for this reason is far from uniform in composition. Since blood pressure of ventricular origin is lower in them than in arteries they are more subject to differences in gravity pressure. An instructive contrast in structural adaptations is afforded by the coronary veins of the heart and the veins which carry blood from the foot to the

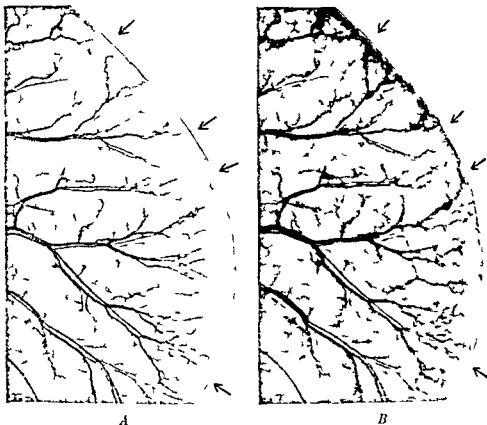


FIG. 46 — Demonstration that the dye Chicago blue escapes from blood stream into tissues more easily from venules than from capillaries. *A* Ear of living mouse photographed in oil thirty seconds after intravenous injection of the dye. The arrows point to the arteries which are thin and straight. *B* Same five minutes later. Same arteries identified by the arrows. They pass to capillary supplied areas which are still unstained whereas coloration about the venules is well established.  $\times 13$  (Smith and Rous courtesy of J. Exp. Med.)

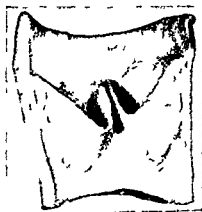


FIG. 47 — Valve in saphenous vein (from Edwards and Edwards, courtesy of Am. Heart J.)

right auricle In the first the force of gravity is not a factor demanding compensation and the distance is short, in the second gravity is a potent deterrent to blood flow and the distance is long.

Heller's (1942) paper, on circulation in normal and varicose veins, merits careful reading. Briefly stated the circulation time from foot to throat is as much as six times slower when the body is vertical than when it is horizontal and the time is very greatly reduced, when in the vertical position, by movement of the leg Two structural mechanism help the venous return The first is the development of valves in the veins so that, when the column of blood is shifted, it can only move upward (see Fig 47). The second is the anatomical relationship of muscles to veins By their contraction the soleus and gastrocnemius muscles, particularly, and the muscles of the abdominal wall, press on the veins which became engorged with blood when the muscles were relaxed.

**Special Adaptations.**—This general correlation of form and function in the 6 principal types of blood vessels just skims the surface Each and every vessel is an individual conditioned by heredity and shaped by environment's specific demands.

Williams (1933) gives a digest of evidence from identical twins. Reference here is made to only one pair "For forty years these two women lived amid very different sets of environmental influences One had occupied herself with factory and farm work The other had directed the affairs of a quiet, urban household At the age of sixty-six, the first developed a systolic blood pressure of 175 to 178. Almost simultaneously the other's pressure rose to 175 to 182. A little over two years later, their symptoms disappeared and their pressures fell to 140 mm and 130 mm respectively " The hereditary endowment being equal, the blood vessels involved in hypertension, underwent similar changes

The strain placed on individual vessels, by the service demanded and the environment in which they live, is far from uniform One factor productive of diversity is distance Where the circuit from ventricle to auricle is long (extremities) and short (coronary circulation) the blood pressure and speed must be dampened down to about the same degree for the capillary bed to function and differences are to be expected in some at least of the types of vessels involved Thus, the intimal and medial changes in radial arteries at sixty-five years are not further advanced than in coronary arteries at twenty years (Cohn, 1942) To put it differently, the coronaries suffer far more in the line of duty Another factor is constancy of service The cerebral arteries supply a tissue the volume of which remains fairly constant, the uterine arteries supply an organ which changes in size enormously with each pregnancy. Their walls are therefore of very different structure. The coiled arteries of the uterine mucosa (p. 357) and the helicine arteries of the penis (p. 334) have special duties to perform.

Even for the same artery the mechanical problems, which appear at first view to be similar, are sometimes solved in different ways by different species In the human pulmonary artery the muscle fibers are circular, oblique and longitudinal; in the guinea pig and ox, spiral, while in the cat there are 2 layers, inner circular and outer longitudinal

In some regions the blood gushes directly from arteries into veins by *arterio-venous anastomoses* entirely sidestepping the capillaries These short cuts are found by Grant and Bland (1930) to be particularly numerous in the palms of the hands, the soles of the feet and the ends of the digits, where their average diameter is 35 microns They are apparently absent in the lobe of the ear It may be, as the

authors say that very special demands are made on the blood vascular system in the extremities which are further removed than the ears to protect against cold. Arteriovenous anastomoses are however known to exist in other parts of the body in which the regulation of temperature would not appear to be their primary function as for example in sympathetic ganglia (Nonidez 1942), in the glomus coecum and in association with the Pacinian corpuscles. A review of the literature on glomus and glomus tumors is supplied by Ottley (1941-42). The cavernous spaces of the penis, clitoris and nasal tunica propria are arteriovenous anastomoses concerned with erection (p. 357).

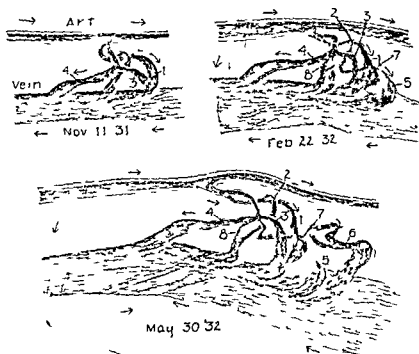
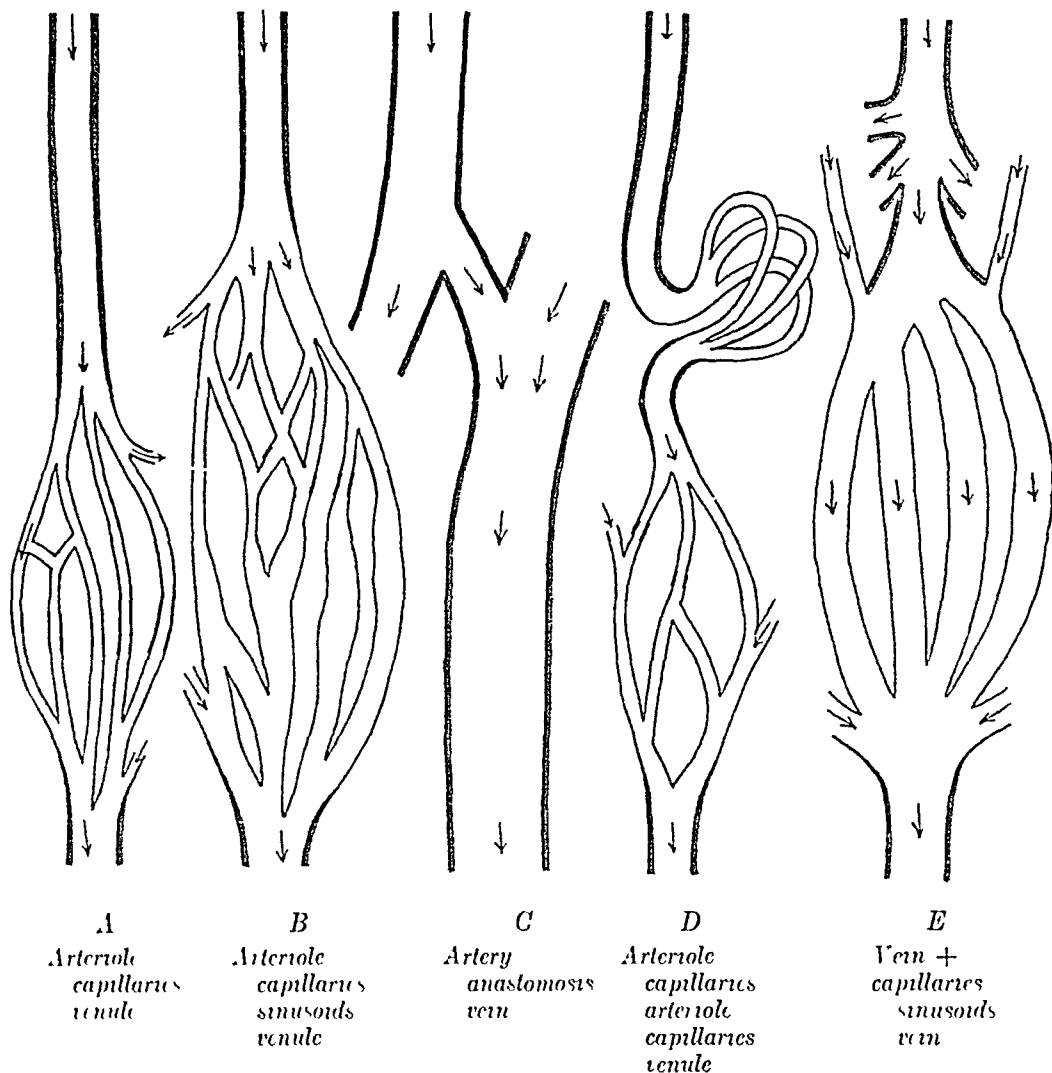


FIG. 48.—Three successive camera lucida drawings of the same group of arteriovenous anastomoses from a table performed tissue chamber installed in ear July 28, 1931. The first sketch (November 11) shows original group of four AVAs (Nos. 1, 2, 3, 4) all branches of same artery present since operation (three and one-half months previously). February 22 (seven months after operation) three additional AVAs have appeared—Nos. 5, 6, 7 and 8 both branches from the original artery and No. 5 from another artery beneath the vein. May 30 (ten months after operation) another new AVA (No. 6) arising from the deeper artery has appeared. All others of original group still present.  $\times 79$  (From Clark, E. R. and E. L. courtesy of Am. J. Anat.)

Many details in the new formation and behavior of individual arteriovenous anastomoses directly observed in the living animal over long periods of time are supplied by the Clarks (1934a). Figure 48 gives a dynamic conception of these peculiar vessels by showing how they develop and flood the veins with arterial blood.

Atypical capillaries are found in many tissues and organs. They are the creatures of the blood stream on one side and of their particular tissue fluid environments on the other despite the fact that the tissue fluid may be present in such small amounts as to escape notice. Some special adaptations are illustrated dis-

grammatically in figure 49 (A) The intervening vessels between an arteriole and a venule are usually all capillaries (B) But the capillaries near the venous overflow may become enlarged and sinusoidal as in the reticular layer of the adrenal cortex (C) Capillaries may be completely dispensed with and the blood surge





small contributions of blood from nutritive capillaries formed by branches of the hepatic artery at the periphery of the lobules.

Venules are probably finely adjusted in an individual way to the service performed but it is in the veins that the most marked individuality is apparent. Among veins entirely devoid of smooth muscle in their walls Maximow and Bloom (1942) list those of the spinal pia mater of the retina, the sinuses of the dura mater and the trabeculae of the spleen. Muscles when present may be arranged differently in the same veins of different species. Thus intrahepatic throttling veins occur in only 3 of more than 30 kinds of mammals studied (Arey, 1941). In raccoons the muscle is distributed in prominent evenly spaced rings. In seals it is chiefly in ring formation, but the rings are incomplete or branched in slanting connections giving the impression of a spiral. While in dogs the arrangement varies from an imperfect spiral through a loose perfect spiral to rings. Spirals and rings of muscle can not only act as throttling mechanisms but by timed contraction can promote a milking action. In several veins such as the human suprarenal the muscle is chiefly longitudinal or placed at an acute angle with the long axis. Reduction in venous length rather than in birth would appear to be its principal function. For other interesting regional adaptations see Liss (1940) comparison of the portal vein and the inferior vena cava.

### SUMMARY

*Elastic arteries spring from the heart.* They include the innominate, subclavian, proximal parts of the common carotids and the pulmonary. Elastic fibers predominate in their medial layer and they extend peripherally to points where the media is mostly muscular. These elastic arteries first receive the blood expelled by ventricular contractions. Because they are elastic and owing to peripheral resistance to blood flow they expand and the elastic recoil of their walls continues to force the blood onward in the intervals of ventricular relaxation. End-diastolic expansion is partly limited by a restraining adventitia in which there are many collagenic fibers.

*Muscular arteries* — These are the continuations and branches of the elastic arteries. Their medial layer is very muscular and contains few elastic fibers. They reach to the little arteries or arterioles which latter are arbitrarily considered to be just beyond the limits of naked eye visibility when viewed *in vivo*. Muscular arteries distribute the blood to the various organs. Their girth is more under nervous and hormonal control than that of the elastic arteries. In traumatic arterial spasm their lumen can be completely occluded. When more blood is required as during pregnancy they can enlarge several times.

*Arterioles* extend from the muscular arteries to the capillaries. In the gradual transition from muscular artery to arteriole the proportion of muscle in the vessel wall increases so that arterioles are for their size more muscular than any other kind of blood vessel. Consequently it is at this level in the circulation that nervous and hormonal factors are most operative. In the transition to capillaries typical circular muscle fibers are lost. Arterioles provide sufficient peripheral resistance to blood flow to iron out pulsation so that blood enters the capillary bed in even streams. By contraction they further increase peripheral resistance and therefore arterial blood pressure. By relaxation they let more blood into the capillaries. When this is carried to an extreme the outrushing blood quickly leaves the capillaries and accumulates in the veins, particularly the abdominal ones. Arterial blood pressure falls and shock ensues.

*Capillaries* are almost naked endothelial tubes devoid of muscular or connective tissue fibers, to which only a few scattered pericapillary cells may be applied some of which are contractile (Rouget cells). Capillaries are the "physiological" sector of the circulation where the exchange between blood and tissue fluid chiefly takes place. They are least under direct nervous control.

*Venules* drain the blood from the capillary bed and are first marked by a patchy investment of connective tissue cells and fibers. They are highly permeable. As they increase in size a little muscle appears.

*Veins* are formed by the confluence of venules to form vessels sufficiently large to be seen in the living state with the naked eye. They possess an intima, a very rudimentary media containing many elastic tissue fibers, some muscle and a few collagenic fibers. Since the blood pressure in them is low the return of blood is facilitated, particularly in the long veins of the extremities, by valves permitting passage only toward the heart and by the massaging action of nearby muscles.

*Arteriovenous anastomoses* are short cuts between arteries and veins which enable the blood stream to by-pass the capillary bed. Their muscular walls are under nervous control.

## CHAPTER V

### HEART

THE heart is a pump designed to force blood through two circuits under low and high pressure respectively. The first is the pulmonary circulation providing for aeration and is represented in figure 1. The second is the systemic circulation carrying oxygen to all parts of the body including the lungs under a sort of forced draft and is indicated below the heart in the same figure. Accordingly the heart is divided into two parts which can be regarded as light duty and heavy duty engines.

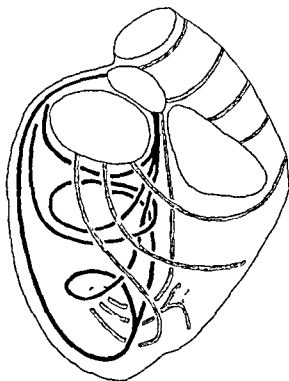


FIG. 30.—Diagram showing the course of the chief muscle bundles. The musculospiral group of fibers are in solid black and the sinospiral in shading. The bundles immediately around the left ostium and the conus form single loops which attach themselves to the aortic septum. All other bundles may be considered modifications of these two simple loops. (Mall, *Am. J. Anat.*)

The right heart is made up of the right auricle which receives venous blood and of the right ventricle which forces this on into the low pressure pulmonary circulation. The left heart is built on the same plan, the left auricle receiving the oxygenated blood from the lungs and the left ventricle forcing it out to the body as a whole under high pressure. The direction of flow is controlled by valves and the four chambers of the heart, like the cylinders of an engine, are accurately timed. The auricles pass the blood into the ventricles at the very moment the ventricles are ready to receive it.

From both the embryological and evolutionary points of view the heart is a blood vessel which has developed chambers, valves and regulatory apparatus.

enabling it to function as a propulsive mechanism. The same histological building materials are used: endothelium, as a lining; collagenic and elastic fibers, for support, muscle, for power, nerve fibers, vasa vasorum and lymphatics for regulation and maintenance.

**Endocardium.**—This internal lining consists of endothelium and a variable complement of underlying connective tissue, nerves, blood vessels and lymphatics. Purkinje fibers may be encountered in it and are recognizable by the large size and blunt ends of the individual cells, small nucleocytoplasmic ratio, restriction of myofibrils to peripheral cytoplasm, etc (p. 315)

**Myocardium.**—The muscle fibers are grouped in broad bands so arranged as to give on contraction maximum discharge of blood from cavities of irregular shape—by no means a simple engineering feat. It is instructive to view Mall's (1911) illustrations showing how the bands can be unwrapped. There are two principal systems, musculospiral and sinospiral (Fig 50). An up-to-date presentation is given by the Robbs (1942). Evidence is accumulating that each ventricular muscle has its own blood supply and is more or less a unit because when damaged, in experiments on animals, a characteristic change results in the electrocardiograms.

A "skeleton," made up of connective tissue cells and fibers, pervades the whole heart but is concentrated in the septum, at the points of origin of the vessels and affords fixed attachments for the valves, and origins and insertions for the muscles. The fibers are lifeless in the sense that they have no intrinsic metabolism. Here is another example of the use which Nature makes of dead material. These fibers are mechanically constructed so as to stand intermittent strain for a long time.

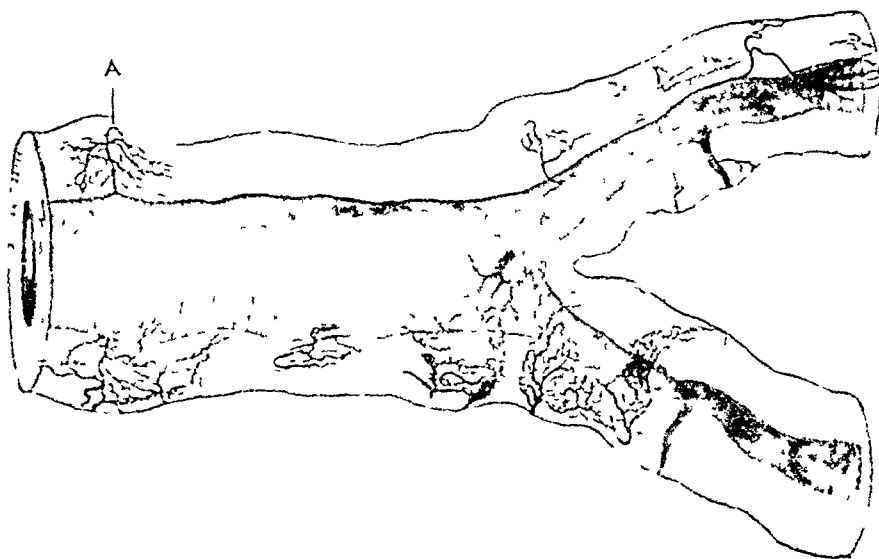


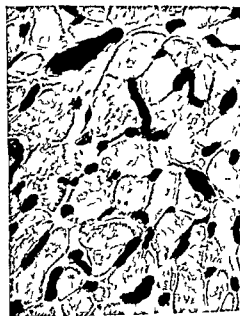
FIG 51—Vasa vasorum of coronary artery, injected with India ink, of forty-two year male. Note "weeping willow tree" pattern at A. (From Winternitz, M. C., Thomas, R. M., and LeCompte, P. M., *The Biology of Arteriosclerosis*, courtesy of Charles C Thomas.)

Since the myocardium is more massive than the muscular wall of any artery, it requires larger vasa vasorum (coronary arteries and veins). Supply, and removal of waste must be uninterrupted. Strain on the coronary arteries is severe. Reference has been made to their precocious ageing (p. 71). Obviously their own muscle, also, has to be nourished. In a man, aged forty-two years having blood pressure of 250/150 and marked cardiac hypertrophy, Winternitz *et al* (1938) found at autopsy

striking enlargement of coronary vasa vasorum (Fig 51). This is in conformity with a rather general increase of vasa vasorum in old and injured vessels whereas in some other tissues (skin) the blood supply tends to decrease. With enlargement of the heart the size of the cardiac muscle fibers and of the capillaries between them both increase as has been well demonstrated by Roberts *et al* (1941) and is shown in figure 52. For structure of the muscle cells see p. 312.



Heart weight 200 gm (age thirty-six years)  
Average fiber diameter 10.5 microns Capillaries 95 per sq mm FC ratio 1.33



Heart weight 900 gm (age thirty-three years) Average fiber diameter 26.5 microns Capillaries 2270 per sq mm FC ratio 1.66

FIG. 52—Size of cardiac muscle fiber and capillaries in relation to cardiac hypertrophy (From Roberts, Wearn, and Boten, courtesy of Am. Heart J.)

Lymphatics which are not very noticeable in the walls of blood vessels are many and well developed in the myocardium. The larger ones as they occur in the dog are represented in figure 53. Fine branches accompany the capillaries in the connective tissue partitions between the cardiac muscle fibers. By contrast the lymphatics in voluntary striated (skeletal) muscle are so poorly developed that they have been described in some muscles as wholly absent.

**Epicardium**—The outermost coat of arteries is the adventitia. There is difference of opinion as to what in the heart corresponds with it. Movement of the external surface of the heart against surrounding tissue is considerable as compared with that of the arteries. Facilitating this movement is a slippery lubricated surface known as the epicardium (*G. epi* upon) made up of a sheet of thin mesothelial cells supported by connective tissue and a little fat.

**Pericardium**—This consists of a layer of mesothelium which is parietal in contrast to the visceral layer that limits the epicardium, backed by a dense fibrous membrane strengthened by special bands of fibers. The architecture of these bands has been worked out by Popa and Iucinescu (1932) and lies more in the domain of gross than of microscopic anatomy. But from the mechanical point of view it is to be remembered that accommodation is afforded for alterations in volume and weight

of the heart within the thorax, which is itself continually changing in shape with each respiration, and is subject to redistribution of pressure with the erect and recumbent position and, further, that the recoil from the forced ejection of blood under high pressure must be taken up.

Because the pericardium is a serous cavity histologically and embryologically like the peritoneal and pleural cavities, there is a mistaken tendency to assume that discoveries made concerning the functional significance of the latter apply also to it. Drinker and Field (1931) have found, by direct experiments, that the pericardium of the rabbit is a singularly inert protective membrane and that the absorption of substances placed within the sac is very much more sluggish than from the other cavities mentioned. "The subepicardial lymphatics are entered with great difficulty from the pericardial sac, a condition favorable to exclusion of the heart from participation in pericardial infections."

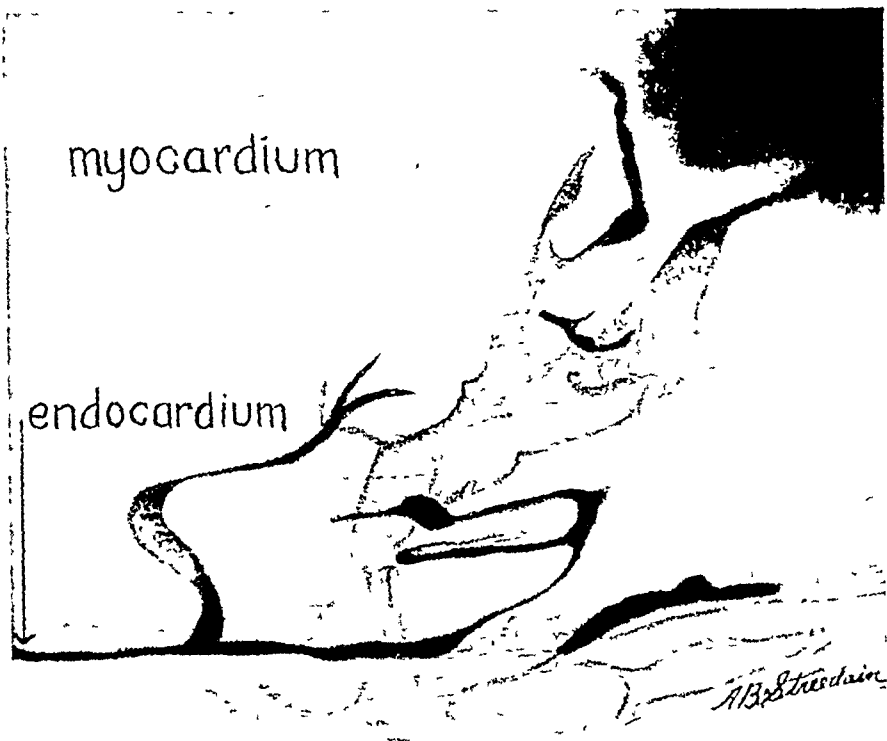


FIG 53 —Lymphatic vessels of myocardium injected with Higgins' American India ink (Dog)  $\times 45$  (From Patek, courtesy of Am J. Anat)

**Valves.**—Valves have been described and figured in veins as structures elaborated to prevent the backward flow of blood (Fig 47). Clearly the need for them is much greater in the cardiac pump. They are greatly reinforced folds of endocardium. Their inner surface is covered with endothelium and their substance is chiefly connective tissue. The supporting fibers are mostly of the collagenic variety and extend from the annulus fibrosus into the valve plate, which, in the case of the atrioventricular valves, is made up of a peculiar chondroid or cartilage-like material composed of small cells embedded in a slightly basophilic ground substance. There are also some elastic fibers but little or no muscle. Consequently the valves have only a feeble blood supply. In attempting to establish the extent of the normal blood supply, valves showing even the slightest signs of inflammation are

to be excluded. According to Dow and Harper (1931-32) vessels occur in the atrioventricular valves but do not extend into them more than 3 mm from the line of attachment. Apparently the semilunar valves are practically avascular. The valves are mechanical devices which rely on physical strength. No structure built chiefly of living elements could stand the strain so long.

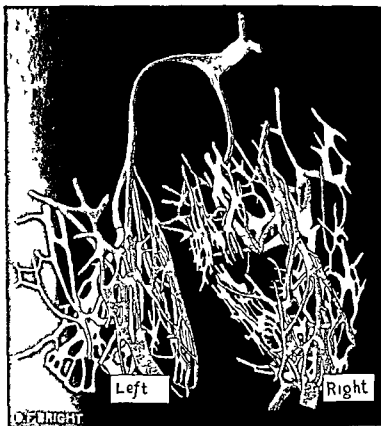


FIG. 31.—Reconstruction of sino-ventricular system of calf's heart (DeWitt L. M. courtesy of *Anatomical Record*)

**Purkinje System**—Made up of the Purkinje cells alluded to, this special conductile mechanism is without parallel in blood vessels. The most complete account is that of Todd (1932). In certain lower vertebrates the cardiac musculature is continuous from the venous to the arterial end of the heart while in man and mammals discontinuity is due to development of a fibrous auriculoventricular septum. It was across this barrier that Purkinje noted the passage of the bundle of fibers which bears his name and is commonly designated 'A-V bundle' and 'bundle of His'. This bundle with its branches in right and left ventricles is illustrated in figure 31. Much attention has been given to it because of the presence of lesions in it in some cases of heart block.

Todd believes that the fibers are not simply a bridge over the septum but extend throughout the heart substance from the entry of the great veins to the terminal expansions of the ventricular walls passing through the atrioventricular junction in diverse manner by several alternate paths. Of this flexiform network through the hazard of chance certain particular areas have received special atten-

tion, namely, the sino-auricular node, the atrioventricular node and the so-called bundle of His. On these alone attention has generally been fixed, to the exclusion of other parts of the system, until popular assumption has endowed these areas with properties which are not their sole requisite but characterize the system in its entirety. The areas mentioned are no different in structure from the remainder of the Purkinje system."

Blood supply is rich and the arteries are said to be terminal in character (end-arteries). By this it is meant that the terminal twigs do not anastomose with those of other vessels so that the blocking of one of them might conceivably result in the complete shutting off of arterial blood from a certain area which is important, if true. Little is known of innervation in man, but in the ox, Scaglia (1927) has discovered numerous myelinated and non-myelinated nerves, ganglion cells and nerve endings, some directly on the Purkinje cells, and nerve cells are abundant along the course of the fibers, which, as one would expect from their myofibril content, are definitely contractile (Wachstein, 1932).

Just how Purkinje fibers regulate the sequence of events in the heart beat is an open question. It starts in the sino-auricular node where they begin and this node is graphically described as the "pace-maker" for the heart. But Todd questions the complete validity of current theories relating to the conduction of the cardiac impulse by them. He says "It is extremely disconcerting to find lesions of the most gross variety in the course of or even throughout the Purkinje system with no corresponding electrocardiographic features. It is equally disconcerting to be unable to discover an anatomical basis for the most flagrantly aberrant electrocardiographic records."

As in the case of the much simpler blood vessels, so also here, different species have their own particular ways of doing things. Though these fibers are well developed in sheep, horses and oxen they are so inconspicuous in human beings and dogs that Glomset (1941) has reached the conclusion that a special muscular conduction does not exist in the hearts of the two last named species. The structure of the Purkinje cells is described on p. 315.

**Maintenance.**—In the case of blood cells continuity of function is provided by prompt replacement of the worn out ones. In the heart replacement of parts is at a minimum. The largest component is muscle. The muscle fibers increase in size when the heart undergoes hypertrophy in response to increased functional demand but the fibers seldom if ever increase in number. Mitoses in them are extremely rare as are also signs of death. Living conditions are unusually favorable because of the regularly imposed intervals of rest. On the basis of 70 beats per minute these intervals added together amount to about fifteen hours per day. Moreover it is logical to assume that the perfection of the lymphatic drainage of the tissue fluid in which they labor is a definite asset. If the elastic and collagenic fibers are of good quality they last for a long time. Nervous and chemical regulation are well managed. See Nomdez (1941) on innervation.

Weller (1933) has pointed out the acquisition by the heart of "certain inherent protective mechanisms which are not equally operative in many other tissues of the body." The first is the pattern of the arterial blood supply. "Not only is there a variable anastomosis between the branches of the right and left coronary arteries, but the number of these connections probably increases as age advances, so that the heart becomes better prepared to withstand occlusive coronary disease in that very period of life when such lesions are prone to occur. Also there is found in



the myocardium a peculiar intermingling of arterioles derived from different arterial branches. Thus it is often seen that the necrosis of infarction following occlusive coronary disease will alternate in distribution with patches of intact muscle. If the injury were concentrated in a single area death would be inevitable. Of all parts of the body the myocardium is served first with arterial blood. The blood reaches it immediately after filtration through the capillaries of the lungs. To this circumstance Weller ascribed its comparative immunity to malignant disease spread by the blood stream. In trichinosis the parasites may become encysted in skeletal muscle in enormous numbers. In the myocardium, however, not a single embryo can be found although as far as size is concerned heart muscle fibers would be adequate yet something prevents this process. Weller also calls attention to the fact that for some unexplained reason syphilitic lesions do not invade the myocardium with the regularity that one would expect from the fact that the root of all internal organs is the most frequently involved.

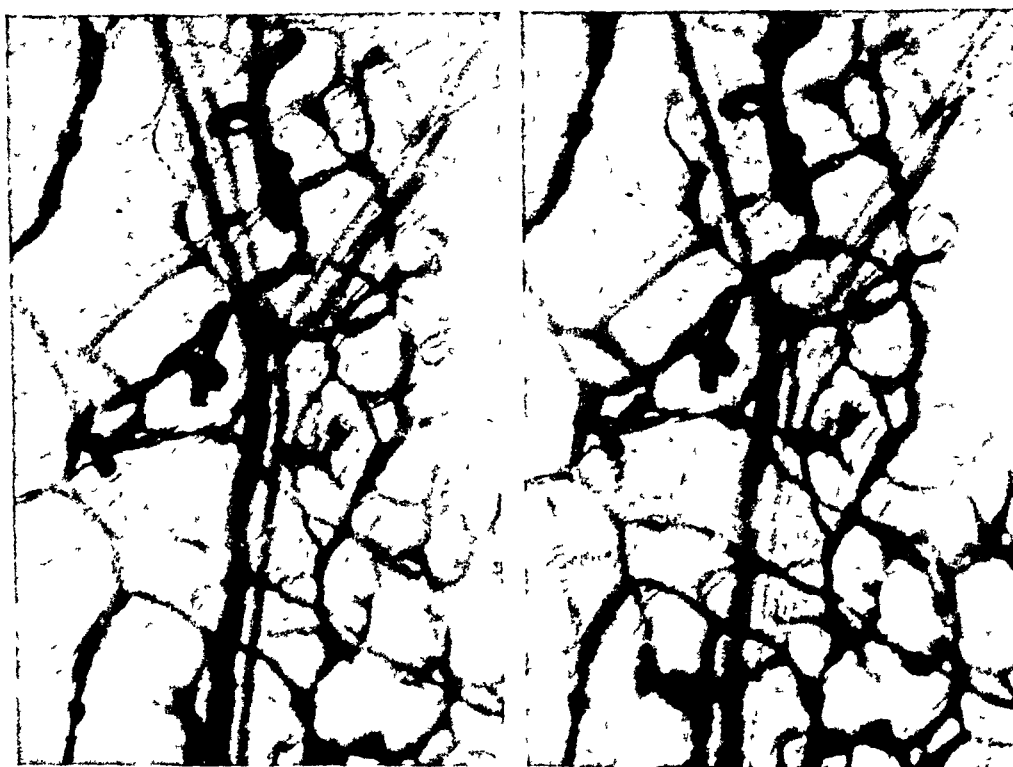
### SUMMARY

The heart is the pump of the vascular system. Its right half serves the low pressure, pulmonary circulation and its left half the high pressure, systemic one. The muscles are attached to the cardiac skeleton which consists of condensation of connective tissue in the septum, at the points of origin of the great vessels at the attachments of valves and in other places. They are disposed in circular bands and spirals arranged so that on contraction they force exactly the right amount of blood out the auricles and ventricles. Direction is controlled by valves. The sequence of events is accurately timed. The entire mechanism is constructed with a view to working for years with hardly any replacement of parts. Friction of the contracting chambers is minimized by the surrounding pericardial fluid and the slippery character of the surfaces. An unusual degree of protection against injuries is provided.

## CHAPTER VI

### LYMPHATIC SYSTEM

BLOOD flows from the heart to the tissues and back to the heart, whereas lymph flows only from the tissues to the great veins at the base of the neck. It is two way water borne traffic compared with one way. The tissue fluids, in which the cells live, are separated from the blood stream by vascular endothelium and from the lymph by still more permeable lymphatic endothelium (Fig 2). Their composition is regulated by what they receive from the blood, by what they give back to the blood, by what they evacuate by the lymph, and by the activities of their cellular inhabitants.



*Hydrokollag*

*Hydrokollag and pituitrin*

FIG 55 —Lymphatics of ear of mouse injected with hydrokollag alone and hydrokollag plus pituitrin. It is evident, in the second case, that the arteriole, which accompanies the vein upward and forks, is constricted while the lymphatics exhibit no response. (Pullinger and Florey, courtesy of the British J. Exp. Path.)

*Lymphatic capillaries* begin in the tissue fluids and gradually converge and unite to form *lymphatic vessels*. Lymphatic tissue (not lymphoid) exists in two forms: Comparatively unorganized mainly as *subepithelial lymphatic tissue* (tonsils, Peyer's patches, etc.) and structurally well organized as *lymph nodes* (not glands). When, in rare cases, these carry blood instead of lymph they are called *hemolymph nodes*. The *spleen* and the *thymus* are special aggregates of lymphatic tissue and will be discussed in a separate chapter.

**Lymphatic Capillaries** — These are naked endothelial tubes which begin blindly (Fig. 2) in those tissue fluids that require more drainage than is supplied by vascular capillaries and venules. In the collapsed condition not carrying lymph lymphatic capillaries are extremely difficult to recognize in histological preparations. The best way to demonstrate them is to inject into the tissue an easily seen fluid such as India ink. This forced addition to the tissue fluid creates an emergency demand for drainage. Fluid and particles enter the lymphatic capillaries which can then be clearly visualized on microscopic examination. A capillary plexus containing hydrokollagen is represented in figure 55. The girth of the capillaries is characteristically uneven. Pullinger and Florey (1935) have shown that while the accompanying arterioles contract under the influence of pituitrin there is no change in the diameter of the lymphatic capillaries which have no muscle.

How fluid gets into lymphatic capillaries has been much debated. One would expect that a bit little lymph they might contain to begin with would be pressed out along the lines of least resistance into the evacuating lymphatic vessels in consequence of the temporary increase in pressure of the surrounding tissue fluid resulting from injections of this sort. But the reverse happens they swell and take in more fluid. Pullinger and Florey have expressed the view that the capillaries are actually pulled open when the tissue is expanded by the increased amount of fluid in it. They have found indications that collagenic and reticular fibers extend between the capillary walls and neighboring structures. With separation of the tissue components these fibers which are not the elastic kind are supposed to exert traction on the capillary walls and to stretch the endothelial cells of which they are made. One can imagine two results of this action. The force tending to draw apart the endothelial cells the flat surfaces of which might be in contact or near to those on the opposite sides of the capillary might slightly lower the pressure in the lumen. The stretched cells and the cement linking them together would be thinner than in the collapsed or partly collapsed state and hence more permeable. Both changes would facilitate the entry of fluid.

A brief survey of the distribution of lymphatic capillaries yields significant data. This great plexiform system of tiny thin walled and thirsty vessels is concentrated under epithelial linings of the digestive respiratory and urinogenital tracts and under the epidermis wherever absorption is likely to occur, or the tissue fluids need supplementary drainage (cf. myocardium). It is lacking in the substance of the central nervous system and in bone marrow, two tissues which are shielded from the external environment as well as in other situations which will be mentioned later (p. 259). It is poorly developed or absent in skeletal muscles.

**Lymphatic Vessels** — As already stated these differ from capillaries in the possession of valves and supporting walls constructed of connective tissue cells and fibers. The larger ones have in addition a little smooth muscle. It is important to observe them in action.

A graphic demonstration of the passage of lymph is obtained by watching the absorption of cream in a cat. A fasting animal is fed a pint of cream and the abdominal cavity is opened a few minutes later under ether anesthesia. At first sight it may be difficult or impossible to detect any lymphatics in the mesentery although a few bean-shaped lymph nodes are visible toward its base and can be felt as firm resistant structures. The abdominal contents should be kept moist with saline and the aperture closed for a time. After a few minutes when again examined the vessels will be clearly marked out in white by the milk fat which has been absorbed by the lacteals and is being transported within them. Careful observation of the

interval between the first appearance of fat and its spread through the vessels in the mesentery will give a rough idea of the rate of flow of the lymph (or chyle) in this region

This should be supplemented by demonstration of a splendid moving picture prepared by Dr Richard L Webb of the Department of Anatomy, University of Illinois College of Medicine entitled "Mesenteric lymphatics, their conduct and the behavior of their valves in the living rat"

Lymph flow resembles that of venous blood insofar that it depends on the pressure of neighboring structures (chiefly contracting muscle) forcing the fluid in a direction determined by the presence of valves. But the flow of lymph is not backed by hydrostatic pressure from the heart and is retarded by the lymph nodes which it must traverse. However the circular muscles in the walls of the larger lymphatic vessels contract more or less rhythmically and serve a propulsive function



FIG. 56 — Mucous membrane over tonsil of white male aged seven years (Tonsillectomy)  
Formalin-Zenker fixation and H & E  $\times 245$

For many reasons ability to identify lymphatic vessels in stained sections is essential. It is a good practice to study places where they are undoubtedly present as for instance the portal canals at the periphery of liver lobules (p. 188). Cross sections of cat intestine, in the phase of fat absorption, stained with Sudan III afford a striking demonstration of lymphatic vessels. Peritonsillar tissue removed at tonsillectomy frequently contains nicely dilated vessels.

Figure 56 shows two vessels beneath the epithelium covering a palatine tonsil. The lower one extends from the bottom of the figure upward and to the right. Its lumen is crowded with lymphocytes and its wall is very thin. The upper one

exhibits a valve and the direction of flow is from left to right. Clumps of lymphocytes, and a heavy deposit of material caused by coagulation of lymph are recognizable in the lumen.

Figure 57 illustrates other lymphatic vessels in the same specimen. One to the right of the center shows coagulated fluid lymph plus a few lymphocytes. This lymph is faintly stained in contrast with the spaces originally occupied by fat which look empty. Some nuclei of the lymphatic endothelium are clearly visible. Similarly arranged nuclei are absent about the fat spaces.

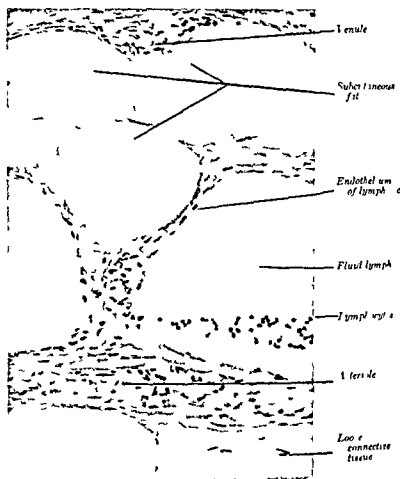


FIG. 57.—Difference between subcutaneous fat and dilated lymphatics in tissue beneath mucous membrane near tonsil. Same case as figure 56. Formalin-Znker fixation and H & E.  $\times 215$ .

**Subepithelial Lymphatic Tissue**—These aggregations of lymphocytes are most highly developed along the alimentary tract where the epithelium is washed by fluids and much absorption takes place.

Among them the *tonsils* hold a prominent place. Not only do they carry a dollar sign, but they are first exposed to all of the in-coming substances. Tonsils are made up of clumps of lymphocytes and lymphocyte-producing cells in a net like matrix of reticular connective tissue. They lie beneath the epithelium and are more or less shielded by it from the contents of the lumen.

Each clump is spoken of as a *lymphatic follicle* (nodule). When active a marked difference exists between the appearance of the outer and inner parts of a follicle. The outer part is deeply stained with hematoxylin owing to the concentration in it of small lymphocytes rich in nuclear chromatin, while the central inner part is less strongly colored because in it typical lymphocytes are scarce and larger cells with more cytoplasm and less nuclear chromatin are numerous. Mitoses occur in all parts of the follicle but they are generally most abundant in the clear center which is therefore called a *germ center* (secondary nodule). In the construction of tonsils the peripheral parts of the several follicles merge with each other while germ centers remain fairly distinct. In old, atrophic tonsils these germ centers are smaller than in young and vigorous ones and may even be absent. Variations in splenic germ centers are shown later in figure 67.



FIG. 58.—Mucous membrane over tonsil. Same case as figure 56. Formalin-Zenker, H & E  $\times 240$ .

In some places the external (distal) surface of the epithelial investment of the tonsil may be smooth while the (proximal) surface projects between the follicles. The follicle, partly shown on the left in figure 58, was at the time of fixation an active producer of lymphocytes as indicated by the fact its pale germ center is so large and is limited only by a few deeply stained lymphocytes in roughly parallel rows. Close examination of this germ center reveals many mitotic figures. There are more lymphocytes about the germ center of the follicle on the right. Some have invaded the epithelial sheet. Observe the close approximation of the follicular tissue to the epithelium.

In other areas the epithelium dips down in between the follicles forming *crypts*. The epithelial walls of these crypts are often thin and may be incomplete as in figure 58 in which a sharp wedge-shaped crypt cuts down into the tonsillar tissue like a knife. It contains escaped lymphocytes and leucocytes. In the depth of the crypt epithelial cells with large faintly staining nuclei and lymphocytes possessed of small intensely stained nuclei appear to be mixed together. Obviously such crypts are places where bacteria can lodge and easily invade the tissue because they are cul-de-sacs and their epithelial walls are so inadequate. Both lymph and lymphocytes are produced in tonsils and are normally carried away by efferent vessels like those already illustrated in figures 56 and 57 which are however especially large by reason of activity of the nearby tonsil.

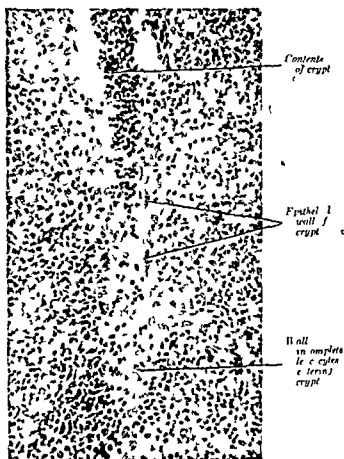


FIG. 9. Crypt in tonsil. Same case as figure 56.  $\times 210$ .

Subepithelial lymphatic tissue is always present in the small intestine where its largest masses are known as *Peyer's patches*. The appendix is well provided with it. But these accumulations do not constitute an equal hazard with the tonsils because the contents of the gut have run the gauntlet of many powerful digestive juices.

Small depots of subepithelial lymphatic tissue normally occur in some other parts of the alimentary tract and more rarely in the respiratory and urinogenital tract but they are normally absent beneath the epidermis where there are nevertheless many lymphatic capillaries.

**Lymph Nodes.**—A good way to describe the structure of a lymph node is to outline, with the help of a diagram (Fig 60), the course of lymph passing through it

Lymph is conducted to the node by *afferent lymphatic vessels* that converge toward its convex surface. This surface is limited by a fairly tough *capsule* made up of connective tissue cells and fibers. Lymph enters through openings in the capsule, usually guarded by valves to prevent backward flow.

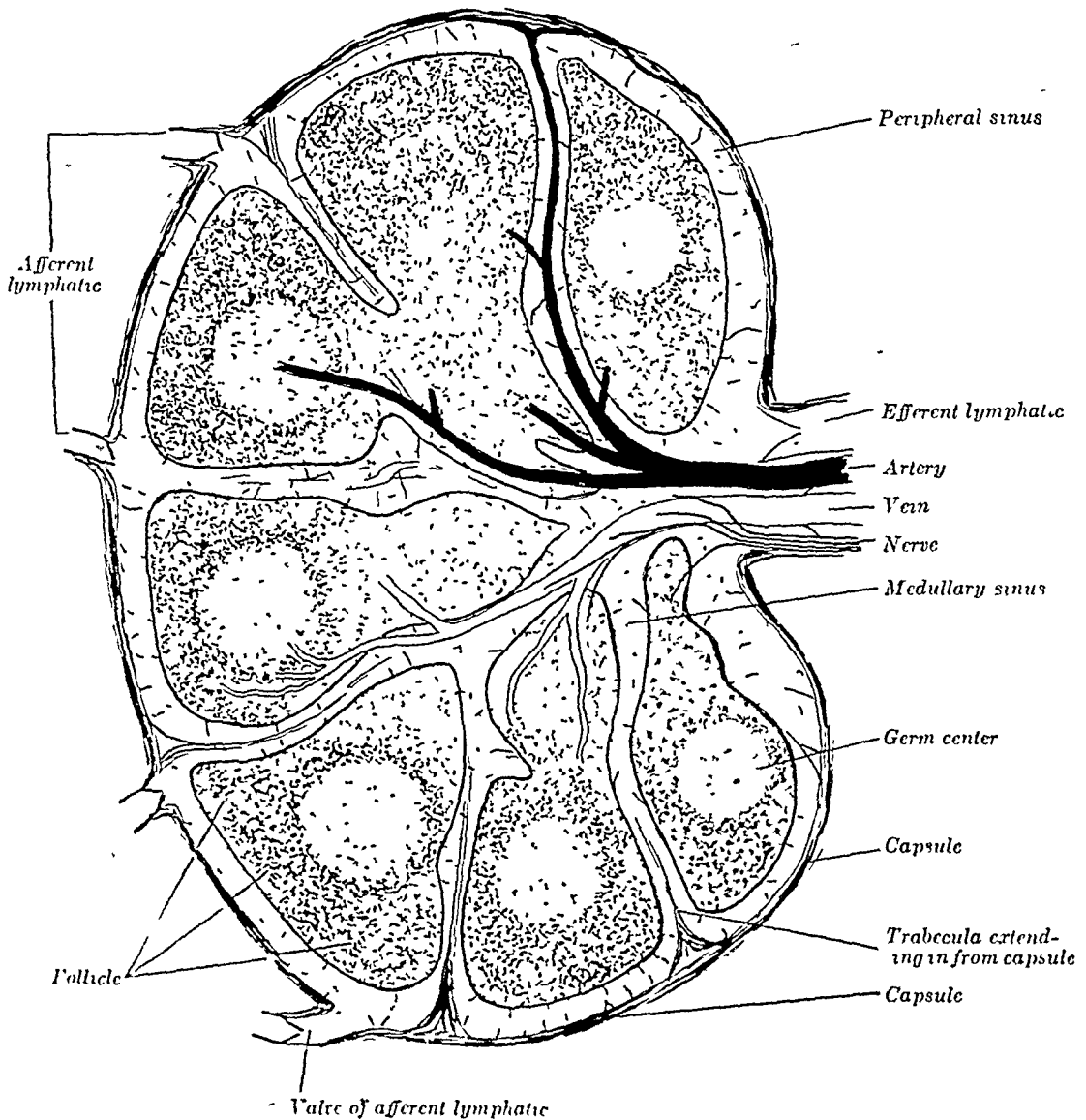


FIG. 60 —Diagram of a typical lymph node. The lymph enters the peripheral sinus by many afferent lymphatics guarded by valves. It percolates in the medullary sinuses between the masses of lymphatic tissue and leaves at the hilus by a large efferent vessel. Arteries (black), veins (clear) and nerves enter at the hilus for supply and regulation.  $\times$  about 20

Within the node the lymph spreads out under the capsule in a space, known as the marginal or *peripheral sinus*, lined by thin endothelial cells, which are more phagocytic than those of lymphatic capillaries and vessels and are therefore called "special endothelial cells." Connective tissue trabeculae (i. e. small beams) stretch



across the sinus anchor the capsule to the reticular framework of the deeper part of the node and serve as pathways for blood vessels and nerves

From this peripheral situation the lymph gradually seeps into the substance of the node in *penetrating sinuses* lined by similar special endothelial cells. These sinuses often accompany the trabeculae. On all sides are lymphatic follicles producing lymphocytes which edge their way into the stream. In case the lymph is comparatively cell free not having come from an area of subepithelial lymphatic tissue or through a lymph node it is here that it first becomes charged with lymphocytes. These follicles are more clearly outlined than those of the tonsils because their extent is limited by the peripheral and penetrating sinuses. Their *germ centers* appear to be favored by direct service with arterial blood via vessels entering at the hilus.

As the lymphatic stream in the penetrating sinuses converges toward the hilus these sinuses occupy relatively more space and are called *medullary sinuses*. Parallel with this change the concentration of lymphocytes in the reticular background of the follicles decreases so that medulla of the node is less intensely colored in histological preparations with hematoxylin than the cortex.

Where all these streams flow together at the hilus they are accommodated in one or more *efferent lymphatic vessels*. The speed of flow is somewhat stepped up owing to reduction in combined cross sectional area of the stream. Protection is afforded by sentinel valves against movement of lymph back into the node when the vessels are massaged by neighboring muscles or contract through activation of their own musculature.

It is difficult to picture construction of more effective lymphatic filters. The fluids poured into lymph nodes have one feature in common that they are produced by the passage of tissue fluid components through the highly permeable walls of lymphatic capillaries. Since the various tissue fluids are not of uniform composition do not all harbor cells of the same kinds and are not equally exposed to extraneous material it follows that the fluids coming from different drainage areas for filtration in regional lymph nodes are themselves far from uniform. Let us consider the fate of lymph-carried particles of different sizes.

Most viruses are of small particle size. Experiments with vaccine virus (Yoffee and Sullivan 1939) show that twelve hours after it is placed in the nose of rabbits it can be detected in the cervical lymph stream. To enter this stream it must traverse at least one lymph node. Virus continues to be spread in the cervical lymph for even days. If other viruses pass through lymph nodes as easily as this lymphatics are a source of danger in the extension of virus infections.

When bacteria penetrate the epithelial defenses and create a disturbance in the tissue fluids they themselves usually accompanied by toxic substances are picked up by lymphatic capillaries and carried to a regional lymph node. Reduction in rate of flow in the node gives opportunity both for the phagocytosis of bacteria and for the toxic substances to act on the node. The destruction of bacteria is fairly effective but the case reports that living organisms persist in nodes for surprisingly long periods of time. Detailed information on this subject and on the lymphatic system as a whole is given by Drinker and Yoffee (1941). The toxic substances may increase capillary permeability increase the tissue fluid in the follicles perhaps stimulate cell division with the result that the nodes swell and become easily palpable. Enlargement is however restricted by the capsule so that the nodes become hard and have been likened in some cases to buckshot.

*Carbon particles* are of variable size, they tend to clump together and lymph nodes filter them out very effectively. At autopsy thoracic lymph nodes are often seen to be blackened from inhaled carbon while abdominal ones, not having filtered

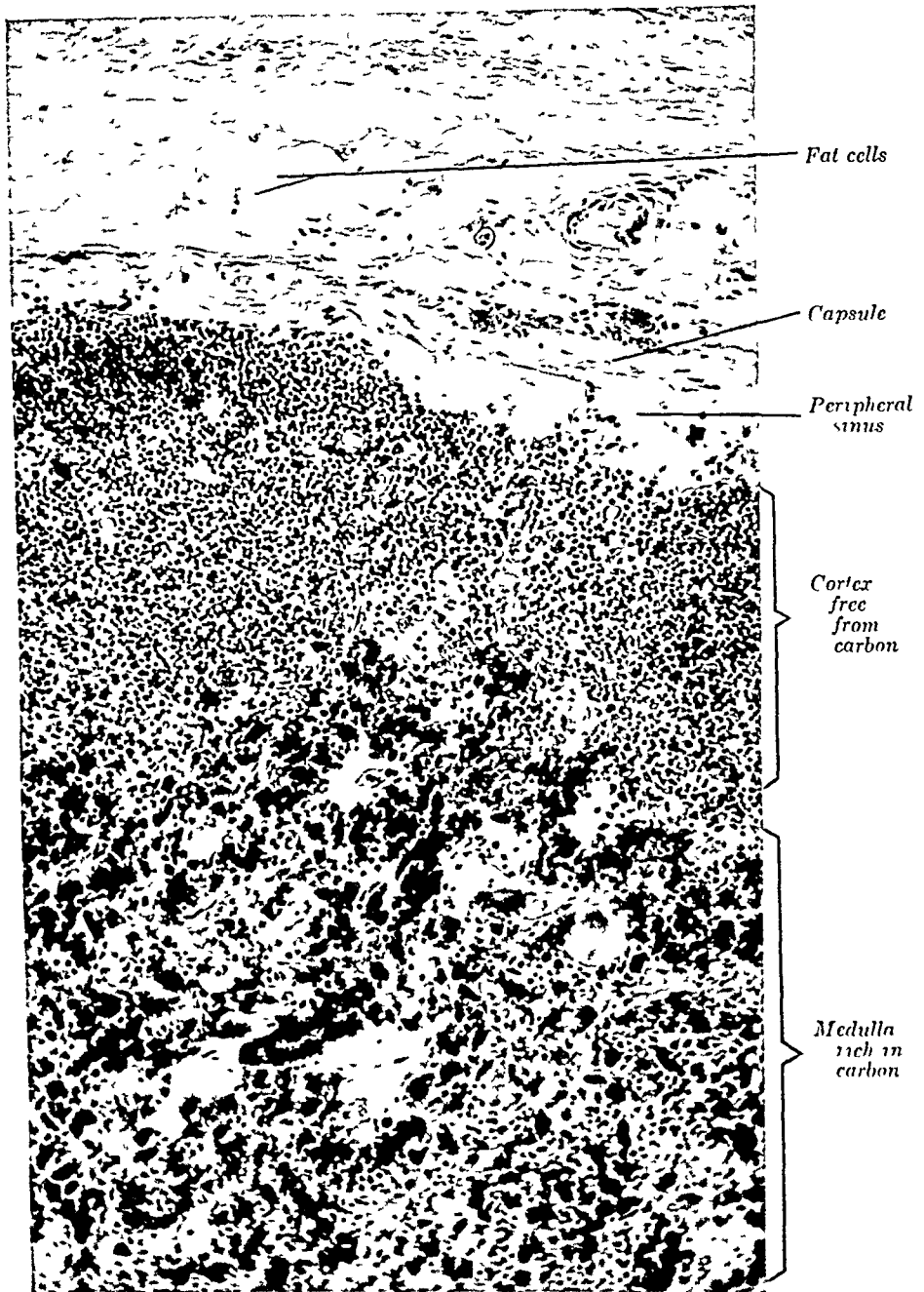


FIG. 61 --Distribution of inhaled carbon in thoracic lymph node of sixty-seven year old white male who died of carcinoma of stomach. Autopsy six hours later. Formalin-Zenker fixation and H & E.  $\times 145$  (Tissue obtained from Dr. R. E. Stowell.)

out carbon, are white. Figure 61 is from a section of the capsule, cortex and part of the medulla of a thoracic node. The carbon is concentrated in the medulla where most of the phagocytic cells are located. A macrophage, containing a fair amount of carbon, is illustrated at higher magnification in figure 62. It is to be

noted that a nearby lymphocyte and neutrophile are devoid of microscopically visible carbon. To pick it up is not their business. In the lower part of the same figure a plasma cell is presented. Cells of this kind are of moderate size, possess a deeply staining, usually eccentrically placed nucleus and basophilic cytoplasm in

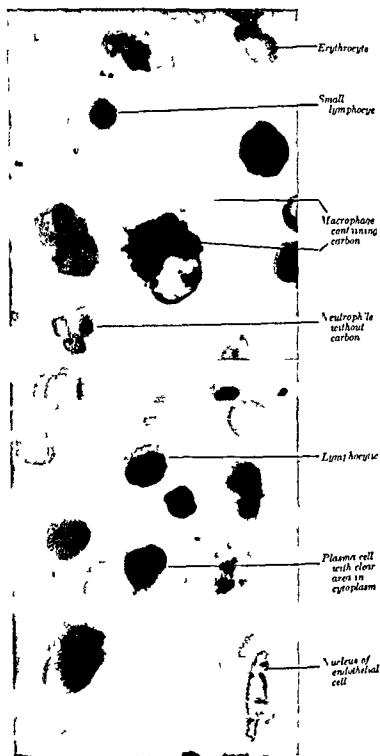


FIG. 62.—Photomicrographs of cells from medulla of same thoracic lymph node as figure 61.  $\times 1500$

which a clear area is recognizable that takes the stain only faintly. They are of lymphocytic derivation and are not phagocytic. What their function is remains a mystery

*Cancer cells* are much larger than bacteria or carbon particles. Unlike them, these cells originate in the tissue fluids so that entry through an epithelial barrier is not involved. That they spread in lymphatic capillaries and vessels is well known. They are arrested in regional lymph nodes (Fig. 63) where they multiply and crowd out the lymphatic tissue. The nodes swell and become palpable. Some cancer cells drift along to the next node and eventually are disgorged into the blood stream. Without these lymphatic streams and filters the spread of cancer would be less. The object of surgeons is to remove the primary growth and the tributary lymphatic vessels and nodes. To examine a volume by Taylor and Nathanson (1942) on "Lymph Node Metastases" is eminently worthwhile.

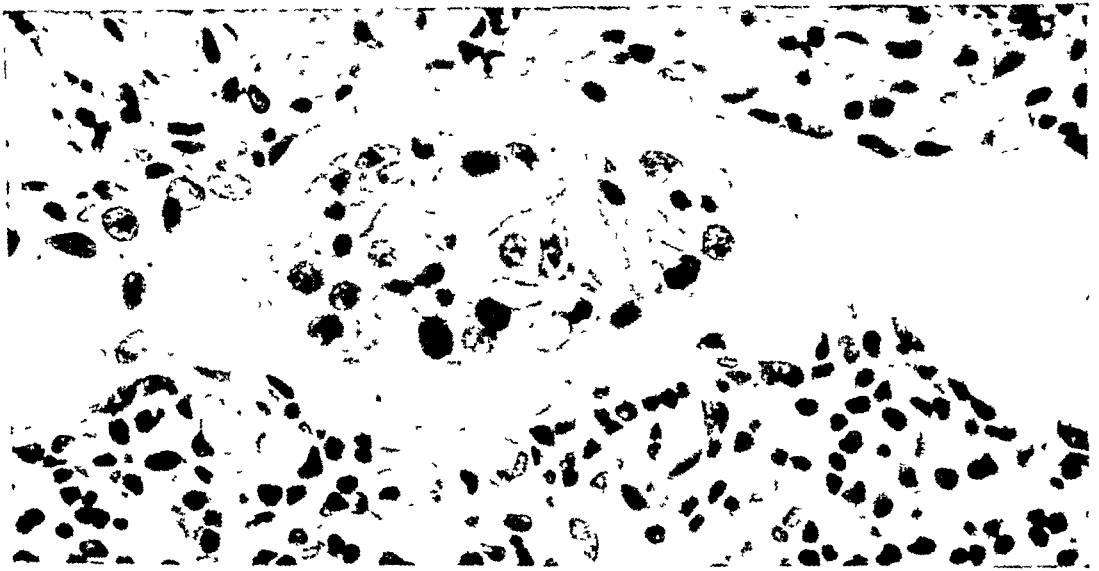


FIG 63 —Adenocarcinoma metastasis in peripheral sinus of retrosigmoid gland from primary in rectum in a man aged fifty-eight years.  $\times 480$ . (Specimen lent by Dr. M. G. Seelig)

The speed with which some materials are drained from the tissue fluids by lymphatics is remarkable. Calcite particles ( $1-2\ \mu$ ), injected intravenously in dogs, have been reported in the thoracic duct lymph twenty-five minutes later while microfilariae ( $40 \times 5\ \mu$ ) make the same journey in ten minutes (see Drinker): Think of the obstacles passed!

The rôle of lymph nodes in antibody production has been much discussed. Ehrlich and Harris (1912) have measured the antibody formation in the popliteal lymph node of rabbits after injection of antigens into the foot by determining antibody titer in lymph directly collected from afferent and efferent vessels. They discovered that specific hemolysins and agglutinins are produced beginning two to four days after plantar injection and that the maximum concentration reached by the sixth day represents an increase of 100 times. Correlated with this increase in antibody is a marked increase in lymphocytes, which cells they believe to be chiefly responsible for antibody synthesis. Results of further experimentation are eagerly awaited.

**Evolution** — A prime essential in the organization of the body is the maintenance of tissue fluids in amount and in composition suitable for their cellular inhabitants. Below the vertebrates these fluids are served only by the blood stream. Fishes, amphibians and reptiles are supplied with an additional system of tissue fluid drainage consisting of lymphatic capillaries and vessels. Passage of fluid through the vessels is accelerated by pumping stations known as *lymph hearts*, located at strategic points. In birds lymph hearts are only recognizable in embryos. In adults they are absent and a few lymph nodes are provided in some species. In mammals the number of lymph nodes is greatly increased and direction of flow is regulated by the development of valves.

In her experiments Nature has been daring and willing to pay the Piper when the objective is so important. Though it has been said to indicate that the lymphatic system is not an unmixed blessing. As will be explained later the evolution of higher forms was made possible by introduction of the female sex hormones but in utilizing such substances Nature plays with fire because some of them are definitely carcinogenic.

### SUMMARY

*Lymphatic capillaries* are thin walled plexiform endothelial tubes which exist in greatest concentration beneath the external and internal surfaces of the body where the tissue fluids are most directly exposed to absorbed substances. They are also abundant in heart muscle and certain other tissues. They are absent in some deep lying tissues like the central nervous system and bone marrow. Because lymphatic capillaries are more permeable than blood capillaries they serve to regulate the composition of tissue fluids by removal of substances which cannot leave by the blood capillaries.

*Lymphatic vessels* have thicker walls made up of connective tissue with a little muscle and are provided with valves. Lymph is pushed along by constriction of the vessels and by the massaging action of neighboring muscles.

*Subepithelial lymphatic tissue* consists of groups of lymphatic follicles in exposed situations (tonsils, Peyer's patches). They are drained by lymphatic capillaries and vessels.

*Lymph nodes* are organized masses of lymphatic follicles provided with peripheral penetrating and medullary sinuses enclosed in a capsule. Lymph enters the peripheral sinus by afferent vessels, seeps very slowly through the sinuses and leaves by efferent vessel. The nodes serve as filters and as places for the production of lymphocytes which are also formed in the subepithelial lymphatic tissues.

## CHAPTER VII

### SPECIAL LYMPHATIC ORGANS

**Spleen.**—This organ has 3 claims to distinction. It is the largest mass of lymphatic tissue in the body; in it is the largest accumulation of phagocytic reticulo-endothelial cells and it is the greatest blood filter. Yet it is not essential to life for it does not provide any kind of tissue not present elsewhere.

The first English translation of Pliny contains the following passage. "This member hath a proprietie by itselfe sometimes, To hinder a man's running. where-upon professed runners in the race that bee troubled with the splene, have a devise to burne and wast it with an hot yron. And no marveile for why? they say that the splene may be taken out of the bodie by way of incision, and yet the creature live neverthesse . . ." (Quoted from McNee, 1930-31.) In chronic malaria and certain other infections the spleen may attain enormous dimensions.

The Roman anatomist, Galen, spoke of the spleen as an organ "full of mystery." For him the body fluids were not blood, tissue fluid and lymph, but phlegm, choler and melancholy. The spleen was thought to be the home of the last named. Hence the English—"venting his spleen."

A good introduction to the spleen consists of a demonstration of spleen and lymph nodes removed at autopsy. Such a spleen is considerably shrunken. It is only about one-third of its weight *in vivo* because so much blood has drained out of it into the large, easily distensible, abdominal veins. In comparing spleen and lymph nodes note, first, differences in color, shape, consistency and in the character of the surface. In both the blood supply enters through an indentation—the hilus. The spleen has a peritoneal investment but no afferent lymphatic vessels. In sections of spleen cut with a razor blade observe the white (lymphatic) and the red (blood) pulp between easily recognizable trabeculae extending inward from the capsule and branching. The white pulp consists of rather elongated masses of tissue (say 0.2 x 0.8 mm). These are Malpighian bodies and correspond to the lymphatic follicles of lymph nodes.

**Microscopic Landmarks.**—Figure 64 is a guide. Above, a section of spleen is represented at low magnification and, below, a key to it in outline. The following details may be distinguished in any good hematoxylin and eosin stained section of the spleen.

When the capsule is compared with that of a lymph node, its thin peritoneal coating of flattened mesothelial cells will at once be apparent. The capsule itself is thicker and its components rather different from the limiting membrane of a lymph node. Collagenic fibers are present, as in the capsule of a lymph node, but elastic fibers are relatively more numerous and a few smooth muscle cells occur. The splenic capsule is, therefore, more distensible than the capsule of a lymph node and slightly contractile.

*Trabeculae* (*t*) invade the splenic pulp from the capsule and possess relatively more muscle and elastic fibers than the capsule. Veins (*v*) and arteries (*a*) can be seen in them. Nerve fibers and lymphatics are present, but inconspicuous.

*Malpighian bodies* are readily identified as masses of lymphocytes embedded in a connective tissue reticulum (p. 271). A little to one side of the center of each is a small artery and some of its branches can probably be made out. Some Malpighian bodies are obviously cut at the side and will not contain any so-called "central

artery. Normally they exhibit great variations in size and in activity. About the periphery of each body is a marginal zone (*mz*) containing many capillaries arising from the central artery, and fewer lymphocytes. About this, in turn, is a venous sinus zone in which the sinuses greatly predominate over the capillaries.



FIG. 11. Spleen, boy, aged eight years, hemorrhagic purpura and marked platelet deficiencies. Above, appearance of section; below, key to section. Outlines of lobules indicated by thick lines and defined by distended venous sinuses (*s*). At the center of the follicle is the lymphatic follicle (*f*) surrounded by fairly compact marginal zone (*mz*). The trabeculae (*t*) are seen primarily near the capsule and tend to limit lobules. At *r* is a large trabecular vein and above and to the right a group of arteries (*a*). (MacNeal Contrib. to Med. Sci. George Wahr.)

*Venous sinuses* (*s*) are identifiable (A) by their position, remote from a Malpighian body, or marginal zone if the section includes only the tissue at the side of a Malpighian body; (B) by their lumina, which are often wide and contain much blood; (C) by their peculiar wall, indicated in figure 68, which is not made of common endothelium but of long, narrow endothelial cells, arranged parallel to their longitudinal axis and bulging into the lumina, and (D) by the fact that they are surrounded by red pulp which contains free red blood cells.

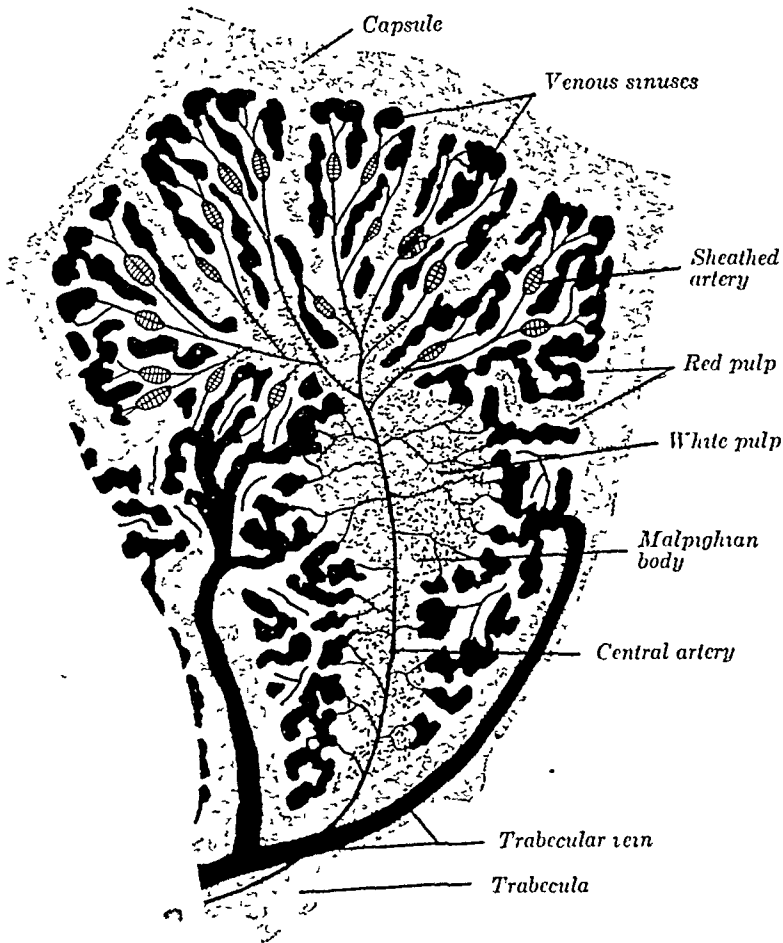


FIG 65.—Diagram of a complete lobule of the spleen (Redrawn and modified from Maximow-Bloom's Histology, courtesy of W B Saunders Company)

**Splenic Lobules.**—These are difficult to define. Their limits are roughly indicated by the broken lines drawn between clumps of distended venous sinuses in figure 64. One is represented, as seen in a single plane, diagrammatically in figure 65. At the base is a broad trabecula containing a trabecular vein, which, by its branches, drains blood from both sides of the lobule. At the core of the lobule is a much smaller central artery which reaches it from the trabecula. This artery runs in a Malpighian body and branches. Some capillaries from it nourish the Malpighian body (white pulp) while arterioles, accompanied by a little lymphatic tissue, extend into the surrounding red pulp. Before these arterioles branch their walls thicken and they are termed "sheathed arteries." After the sheath is lost the arteriole branches and is continued as arterioles, and later as capillaries, into the surrounding



venous sinuses. The periphery of the lobule is marked by them and the trabeculae carrying the trabecular veins that drain them.

**Lymphatic Tissue**—Malpighian bodies resemble the lymphatic nodules of subepithelial lymphatic tissue and lymph nodes in several particulars. (1) They



*Thin germ center, thin lymphocytic border*



*Active germ center, much thicker border*



*Germ center still fairly visible  
though lighter*



*No signs of cell division*

FIG. 6. Variations in Malpighian bodies in the spleens of normal monkeys (*Macacus rhesus*)  
× 90

contain a accumulation of lymphocytes in a matrix of reticular cells and fibers. (2) They exhibit a rim center composed in general of large pale staining cells surrounded by a border of densely packed lymphocytes (Fig. 6a). (3) These reticular producers which enlarge with activity and shrink when effused. But the vascular and lymphatic connections are by no means the same. The Malpighian bodies are held in suspension in what amount

to almost a lake of venous blood (red pulp) without either afferent or efferent lymphatic vessels, whereas the lymphatic follicles have no such investment, are directly drained by lymphatic vessels in subepithelial lymphatic tissue and are surrounded by lymph sinuses in lymph nodes

Lymphocytes, manufactured in the Malpighian bodies, are shed into the boundary zone and migrate into the red pulp. When many of them do this they change the character of the pulp making it less red. They must gain the surface of the trabeculae before they can be picked up by the rather small lymphatic capillaries located in these structures. Perhaps some of them do not travel so far but enter the blood stream through the thin walled vessels of the red pulp. Certainly the lymphatic vessels, leaving at the hilus of the spleen provide surprisingly small drainage for so much lymphatic tissue

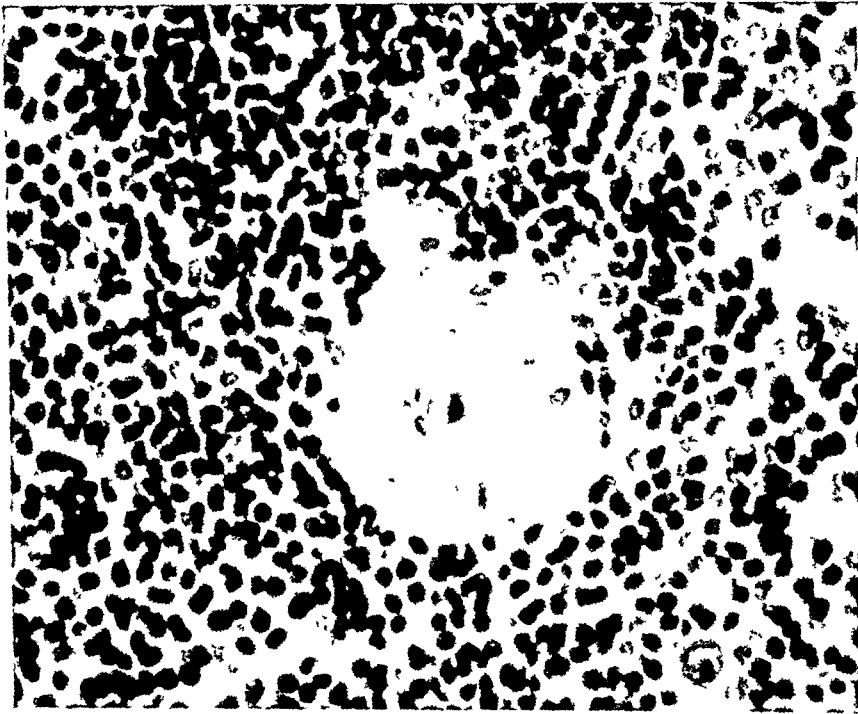


FIG 67.—Semle Malpighian body in spleen of a woman, aged one hundred and eleven years

**Red Pulp.**—Exactly how the blood circulates in the red pulp is not known. Some think that the endothelial walls are incomplete and permit escape of blood cells through openings. Others are of the opinion that they are complete, but so thin that the cells easily pass out and in again. In sections the venous sinuses easily can be seen, likewise rather thick-walled arterioles, called "sheathed arteries" (Fig 68). By the introduction of a new technique, which permits the close study of living spleens of animals by light transmitted through their thin edges, Knisely (1936) has described and pictured the course of blood through the red pulp and filtration by the venous sinuses.

Proceeding from the Malpighian body above and the trabecular vein below in figure 69, he reports 3 sets of sphincters.

- 1 Sheathed arteries placed near the Malpighian body.
- 2 Afferent sphincters located at the beginning of long capillaries that pass directly to the trabecular vein (extreme left in figure) and at the entrance to sausage-shaped venous sinuses (outlined in blue).

3 Effluent sphincters stationed at the far ends of the sinuses near their discharge into the trabecular vein

The filtration is according to Kniesly cyclic and divisible into four phases

*Conduction* — Afferent and effluent sphincters open with lumen narrow wall thick looking and whole blood passing through

*Filtration Filling* — Effluent sphincter closes blood accumulates in the venous sinus which swells some fluid filters out through the wall

*Storage* — Afferent sphincter now closes and some reds also leave the lumen and enter the surrounding tissue fluid

*Emptying* — Effluent sphincter suddenly opens wide and the stored mass of retained blood cells discharges into the vein

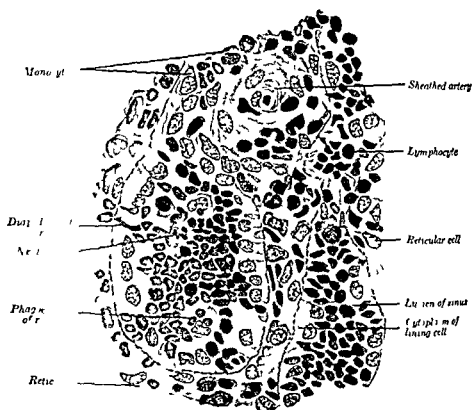


FIG 68 — Spleen  $\times 750$  (Redrawn and modified from Maximow's *Human Histology* courtesy of W B Saunders Company)

Mackenzie Whipple and Wintersteiner (1941) have not been able to confirm Kniesly's findings which he has however demonstrated to the satisfaction of numerous visitors to his laboratory

The main points to be borne in mind are (1) That the amount of blood entering the red pulp is regulated by the afferent arterioles limited segments of which have specially thick sheaths (2) That the exchange of fluid and cells between the blood stream and the tissue fluid is more unhampered than in any other part of the body (3) That in consequence of this blood plasma comes into very intimate relation with extravascular cells and since fluid so easily returns to the blood stream extensive lymphatic drainage is not required (4) That fluids tending to stagnate in the red pulp are pressed out into the trabecular veins by leisurely rhythmic

contractions of the whole spleen made possible by the development of smooth muscle in capsule and trabeculæ

Conditions are ideal for the filtration of blood but only a small fraction of the circulating blood is shunted through the spleen. The amount of blood thus filtered is the quantity required to meet functional demands. If a larger volume were involved the circulation time of the blood as a whole would be increased and a larger, similarly constructed, organ would be required.



FIG 69 Vascular connections between Malpighian body and trabecular venules (Redrawn and modified from Kinsely, courtesy of Anatomical Record)

The materials to be removed naturally differ from those in lymph. Blood is less subject to outside contamination than lymph and the lymph is itself filtered through lymph nodes before it enters the blood stream. The materials sifted out in red pulp are ordinarily those which have first passed the gauntlet of the lymphatics plus others normally resident in the blood stream. The most conspicuous of the latter are blood cells which are taken up by macrophages in much larger numbers. Their cytoplasm becomes charged with an easily identifiable iron containing pigment, *hemosiderin*, formed by the digestion of hemoglobin.

The exact source of these macrophages which also ingest bacteria and other substances, is of minor importance in comparison with what they do. Reference

has already been made to the fact that by the careful selection of cells a series of what appear to be transitional forms between monocytes and macrophages can be established. Here in the spleen and also in lymph nodes two other series of cells of equal value (or lack of it) can be chosen extending between reticular cells and macrophages and between endothelial cells and macrophages. Cells that show this capacity for particulate matter have been dubbed *reticulo-endothelial cells*. Though the spleen contains them in the highest concentration they occur in many other parts of the body and are commonly said to constitute the reticulo-endothelial system.

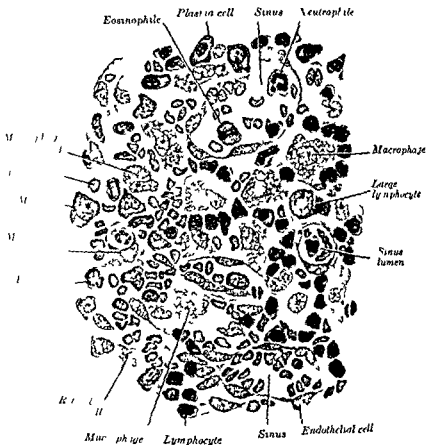


FIG. 70. Red pulp of rabbit spleen. Eosinazure stain (Maximow Bloom Textbook of Histology W. B. Saunders Company.)

**Reticulo endothelial System**—The best way to conduct a preliminary survey of the system is to repeat in small way experiments by the pioneers.

In 1906 Nicolle and Mesnil discovered a dye which they called trypan blue because they found that like the trypan red of Ehrlich it would be helpful in curing trypanosome infections (trypan, anger + soma body—parasites equipped with an undulating membrane giving them anger-shaped bodies). Following a brief report by Boufard Goldmann (1909) at the suggestion of Ehrlich made a detailed study of the distribution of the dye in various animal. Goldmann's work should be repeated by the students using his excellent plates as a guide and in the light of investigations on the chemistry of trypan blue and other dyes by Evans and Schulemann (1914), and Evans and Scott (1921). Each animal given the white mice should be given intraperitoneally 1 cc. of 0.5 per cent trypan blue in sterile distilled water and in the course of a few minutes—the beginning area of the dye in the ears observed. Similar doses should be administered every second day.

for eight days. A few hours after the last the students should draw fresh blood from the tail. They will probably find that the monocytes have taken up a little dye. Then they should autopsy the mice so that they can determine for themselves its distribution in the tissues. Gross inspection shows that the skin, kidneys, adrenals, spleen, liver and bone marrow are quite deeply stained, whereas the central nervous system seems to have escaped. But the heaviest accumulation of dye is in the peritoneum near the site of injection and in the loose connective tissue everywhere. The examination of fresh mounts of tissue in normal saline reveals that the stain is concentrated within: (1) the epithelial cells of the convoluted tubules of the kidney, of the suprarenal and choroid plexus, (2) certain cells of the ovary and testicle; (3) the macrophages of loose connective tissue throughout the body and especially in those of the spleen, liver, bone marrow, adrenals and lymph nodes—fibroblasts are colored less deeply; and (4) in the "specific endothelia" of the five organs mentioned.

Even though stainable by vital dyes epithelial cells do not belong to this system. To be concise it is made up of:

1. Free monocytes and macrophages, wherever they may be. (Perhaps the cells mentioned in the ovaries and testicles are related to them.)

2. Fixed special endothelial cells of lymph nodes, spleen, liver, bone marrow, adrenals, and to a limited extent of the anterior lobe of the pituitary. These cells constitute the walls of lymph sinuses of lymph nodes where the flow of lymph is slow. In the other organs they line vascular capillaries (sinusoids in the liver and venous sinuses in the spleen) where the flow of blood is likewise retarded providing a good opportunity for contact and phagocytosis. These cells, and the reticular connective tissue cells may enlarge, break loose from their moorings and act as free R.E. cells.

Any person trying to list *all* of the functions of the reticulo-endothelial system is simply inviting controversy, but a summary by Wiseman and Doan (1942) is useful. See also the Quarterly Cum. Index Medicus for papers constantly accumulating on the subject. The system certainly plays an essential part in the destruction of red blood cells and hence in bile pigment formation and iron metabolism. It also functions in defense of the body against invading microorganisms and substances of wide variety that may come in contact with its phagocytic cells in the tissue fluids, lymph and blood stream. Moreover the reticular and special endothelial cells retain the potency of blood cell formation on demand especially in the spleen, which is active in this respect in embryos.

## THYMUS

A prominent feature of the thymus is its elusiveness. It attains a maximum weight of approximately 30 grams about puberty after which it becomes inconspicuous. The epithelial components, which it receives from the endodermal pharyngeal pouches, become submerged by hordes of lymphocytes. Opinion swings to and fro as to whether it produces a definite internal secretion, as epithelial organs of similar origin have the habit of doing. It is even difficult to decide whether the thymus is an essential part of the body, because, owing to its position in the thorax, complete removal is difficult without serious injury to other organs. And one has to be on the lookout for accessory thymic tissue (see Van Dyke, 1941). But we do know that the thymus of calves and lambs is good to eat and frequently appears on the table as "neck sweetbread" in contrast to "stomach sweetbread," the pancreas. It is the "enigmatic organ" of the ancients. The name we use is taken from the Latin term, *Thymus*, a genus of plants with a two-lipped calyx, and serves to remind us of its two main lobes.

**Young and Old**—The thymus of an infant looks so different from that of an adult that at first sight one might think that they were different organs rather than the same organ at different ages.

A portion of a thymus of a six and one-half month old baby is represented in figure 71. The lobes are divided into lobules by loose connective tissue partitions extending inward from a thin capsule as every good housewife knows for it is customary to pull away most of the connective tissue before cooking. In these lobules it is easy to recognize a deeply staining cortex and a less intensely staining medulla.

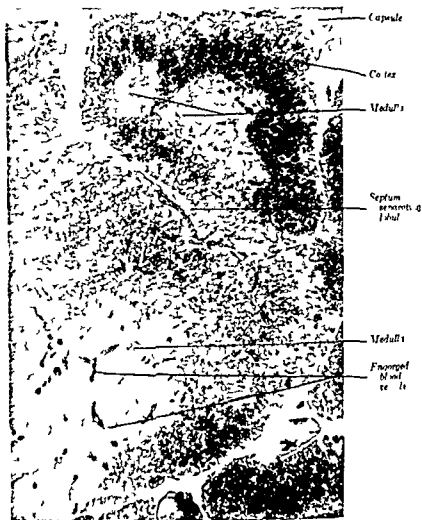


FIG. 71. Infant thymus, fixed in formalin Zenker and stained with H & E from a six and one-half month old baby.  $\times 41$ . (Specimen lent by Dr. J. H. Van Dyke.)

The cortex looks a little like the cortex of a lymph node. Both are crowded with lymphocytes (thymocytes) but in the thymus there is no peripheral lymph sinus. Thymic nodules, in common with Malpighian bodies of the spleen, have no afferent lymphatic vessels of any kind. They are not filters of lymph. The lymphocytes exist in a background of reticular cells and fibers. Reticular cells are said to be of two sorts, mesenchymal and endodermal (epithelial) but it is difficult to find more than one variety marked by a large elongate pale-staining nucleus and considerable cytoplasm.

The *medulla* is generally faintly stained for the same reason that the germ centers of lymphatic follicles seem pale, namely, that deeply staining lymphocytes are much less numerous in them than in the surrounding cortex, but the similarity is only a superficial one. Thymic nodules may be so cut in sections that the medulla seems to exist (like the germ centers) in island formations surrounded by cortex as in the upper part of figure 71. This appearance is however illusory for the medulla extends out into the lobules as cores from the deeper parts of the gland a suggestion of which extension is seen below in the same figure. The medulla is but a poor producer of lymphocytes. But reticular cells and fibers are more noticeable in it than in the cortex and some at least of the reticular cells are of epithelial nature.

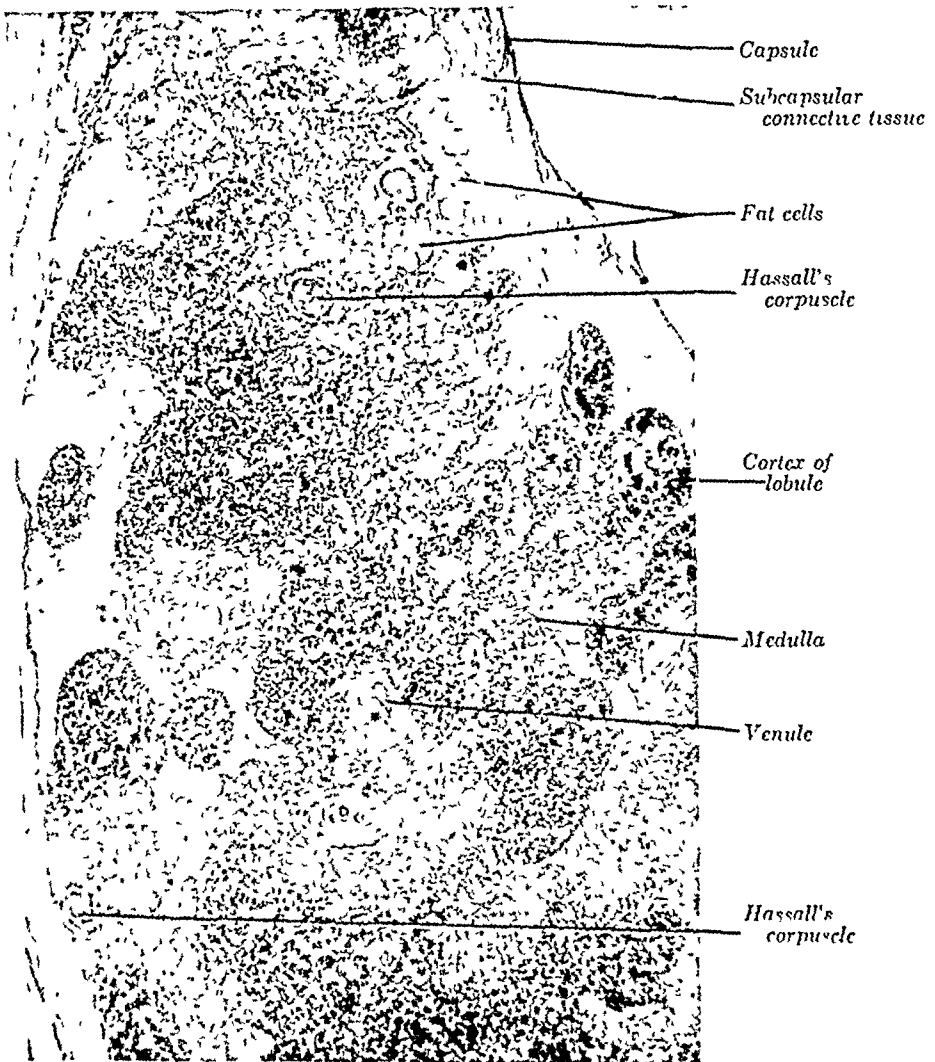


FIG. 72 - Adult thymus showing extensive involution. Formalin-Zenker fixation, H & E.  $\times 41$

By contrast, the thymus of an adult is a shrivelled looking tissue. Its full blown, luscious appearance has departed. The capsule looks thicker, because there has been an increase in subcapsular connective tissue. Under the capsule numerous fat cells have come to encroach on the space originally taken up by the lymphocyte-rich cortex. Consequently the cortical parts of the lobules are much smaller. In them the lymphocytes are more spread out and less closely packed together.



In volume the medulla has also decreased considerably. Many of its epithelial cells are grouped in concentric masses called *Hassall's corpuscles* in the centers of which the cells develop a horny material (keratin) and die. These corpuscles are distinctive constituents of the thymus. But Kent (1937) has produced similar structures by experimentally displacing nests of epidermal cells into muscle in

COMPARISON OF LYMPHATIC ORGANS

	<i>Subepithelial lymphoid tissue</i>	<i>Lymph nodes</i>	<i>Spleen</i>	<i>Thymus</i>
	Not sharply marked capsule absent	Convex except at hilus cap- sule mostly collagenic	Smooth most of fibrous and mus- cular capsule covered by peritoneum	Much lobulated excavated in places by con- nective tissue and fat
	Absent	Present	Present	Absent
	Stratified squamous (over tonsil) sim- ple columnar (over Peyer's patches)	Absent	Absent	As scattered cells and Hassall's corpuscles
	Present	Present	Present	Absent
	Production active	Production active	Production slight	Production very slight
	Efferent	Afferent and efferent	Efferent limited to capsule and trabeculae	Small efferent
Invaded by	None	Large periph- eral penetrat- ing and med- ullary	None	None
Muscle	Absent	Absent	In capsule and trabeculae	Absent
Red pulp	Absent	Absent	Present contains ellipsoids and characteristic venous sinuses	Absent
Cortex and medulla	Absent	Present	Absent	Present
Age of maximum development	Infancy	Puberty	After puberty	Before puberty

which situation the cells clump together and undergo central keratinization. He has also very properly called attention to the likeness existing between Hassall's corpuscles and the epithelial pearls of skin cancers. In the formation of the latter epidermal cells move away from the surface, associate themselves in clumps and similarly become keratinized. This striking resemblance between Hassall's corpuscles and concentric cellular masses of epidermal origin leads Kent to suggest the possibility that the Hassall's corpuscles usually considered to be of endodermal

lineage, are likewise epidermal derivatives, in other words that they are ectodermal, not endodermal

**Function.**—As the thymus atrophies the need for nourishment decreases and its vascularity is reduced. Alterations in blood vessels and reticular fibers are described by Smith and Ireland (1941). Riddle (1935) speaks of the low-ebb thymus of human beings in contrast with its highly developed thymus of birds. There is evidence that the latter produces a hormone, thymovudin, concerned in the formation of egg albumin and egg shell. Claims by Rowntree and his associates (1936) that the giving of thymus extract to rats through successive generations brings about an extraordinary acceleration of growth and differentiation in young rats have not been substantiated. The captivating idea that the long constructive period of youth in human beings is due to comparative lack of thymus hormone is without support. Indeed the thymus as an endocrine appears to be on the way out as far as human evolution is concerned. The blood cell forming function of the thymus (Kindred, 1940) soon wanes. Its lymphatic component, as long as it lasts, may be more valuable than its vestigial epithelial one. Because these hordes of lymphocytes are segregated in a protected part of the thorax away from the areas and streams of absorption, which make the subepithelial lymphatic tissues and the lymph nodes what they are, does not signify that they are without influence on the cellular population of the body as a whole. Neither are Malpighian bodies of the spleen located in lymphatic absorption lines. Compared with the cellular inhabitants of other lymphatic organs, the lymphocytes of the thymus appear most to be in a kind of back water. They stay put for a time only to disappear earlier than those in other situations. But to assume that they are functionless is unwise.

**Comparison of Lymphatic Organs.**—This is presented in tabular form. The reasons for the differences are functional clues that can be read but imperfectly. How the lymphatic tissues reach peaks of development at different ages and waste away at different rates is described in an interesting way by Kumbhaar (1942).

## SUMMARY

The spleen is a remarkable association of lymphatic tissue producing lymphocytes, but not acting in any way as a lymph filter, with highly vascularized tissue which does serve as a blood filter, but which retains the potency of blood cell production. The two exist in a matrix of reticular connective tissue limited by a capsule invested with peritoneum and supported by strong trabeculae.

The lymphatic tissue is known as white pulp and the highly vascularized tissue as red pulp. The *white pulp* is distributed in masses (Malpighian bodies) each surrounded by red pulp, and without either afferent or efferent lymphatic vessels so that the lymphocytes produced must either be removed by the vessels in the red pulp or traverse the red pulp and be picked up by small lymphatics in or near the trabeculae. The course of the blood stream always is to and through the white pulp to the red pulp and out *via* the trabecular veins.

The *red pulp* is made up of many highly permeable venous sinuses. Blood plasma and some cells pass through the sinus walls into the surrounding tissue fluid. No tissue fluid in any other part of the body is more directly exposed to blood plasma—a fact which has several important physiological consequences. Much fluid returns into the sinuses. Supplementary drainage of tissue fluid by the trabecular lymphatics is but slight. Since the current is slow and hesitating the

opportunity for phagocytosis within and especially without, the venous sinuses is excellent. Reticular cells, endothelial cells, and probably blood monocytes enlarge and produce macrophages. Many red blood cells are phagocytized.

The amount of blood entering the capillaries and venous sinuses is as in other situations, regulated by the arterioles some of which have particularly thick walls. Circulation is so leisurely that it is facilitated by rhythmic contractions of the whole spleen made possible by the presence of smooth muscle in both the capsule and trabeculae. Obviously the spleen can greatly increase in size either rapidly by dilatation of the venous sinuses or slowly by proliferation of the lymphatic tissue.

The thymus is an intimate mixture of scattered epithelial cells and of clumps (Hassall's corpuscles) with many lymphocytes in a background of reticular connective tissue. It has no afferent lymphatic vessels, lymph sinuses or germ centers. It is much broken up into lobes and lobules. Both maximum development and retrogression are earlier than in other lymphatic organs. The thymus is not a filter for lymph. Its lymphocytes are not discharged in large numbers into efferent lymphatic vessels as in the case of subepithelial lymphatic tissue and lymph nodes. The function of the thymus still is unknown, but it is more probably an expression of the dominating lymphatic component than of the recessive epithelial component.

## CHAPTER VIII

### ENDOCRINE SYSTEM

THIS system regulates bodily activities through the operation of a series of broadcasting stations, the tuning in of receivers and the resulting action. The endocrine glands are the stations. Each generates and emits a chemical substance or substances peculiar to it and not conflicting or drowning out those discharged by others. Like radio waves these hormones are all very penetrating. In the body medium, which is water, they easily pass through many obstacles. Produced by endocrine cells, they are first discharged into the immediately surrounding tissue fluid. Then they enter blood capillaries (and perhaps lymphatics), are widely distributed in the blood stream and eventually pass out through the capillary walls into the tissue fluids everywhere. But though all the cells in these fluid environments are exposed to them, in varying degrees of intimacy depending on vascularity, only those constructed as receivers for the particular substance respond by appropriate action. It is emphatically not an integration based on conduction along definite paths as in the nervous system, but one that depends on the building up of sufficient material for broadcasting effective concentrations in all directions.

**Thyroid.**—Like the thymus this organ develops from pharyngeal epithelium. It is not however shrouded in lymphocytes but maintains its epithelial character. Aristotle's designation, *globus hystericus*, tells part of the story because those who have hyperthyroidism are frequently geared up and nervous, while individuals suffering from hypothyroidism are subdued and apathetic. The broadcasted hormone, thyroxin, hastens one of the most universal of cellular activities in the body, namely *oxidation*, so that the receivers are legion. To make it *iodine* is one of the substances required and the amount of hormone manufactured is increased on receipt of *thyrotropic hormone* from the pituitary.

To bridge the gap between gross and microscopic appearance examine a human thyroid or the thyroid of a fairly large mammal. Observe its color and how it feels. Locate some of its vessels and nerves. Attempt to strip off its capsule. After thus exposing the surface examine pieces in water with a hand lens and identify the structural units known as *follicles* (Fig. 73). (The extraglandular lymphatic plexus will probably not be visible unless it has been exposed to India ink, or other material, which easily enters it after injection into the tissue fluid. At a slightly higher magnification the capsule, follicles, lymphatics and blood vessels are illustrated in figure 74.) In thick sections, cut with a razor blade, tease out a few individual follicles. In addition follicles isolated by maceration, should be studied. Jackson (1931) has standardized the procedure. Place pieces of the gland in 3 parts of concentrated hydrochloric acid and 1 part of water for twenty-four hours. Wash thoroughly in at least 10 changes of water and gently separate (Technique, p. 191).

Seen in ordinary hematoxylin and eosin stained sections the outstanding characteristics of the thyroid gland are readily made out. It is a mass of follicles (*L. folliculus*, diminutive of *folius* a bellows), closely knit together by connective tissue and serviced by blood vessels, nerves and lymphatics. The wall of each *follicle* is made up of a single layer of epithelial cells which betrays its surface origin. These cells are all of the same kind but show some variability because they are obviously not all of the same age. To make good occasional loss of cells by death, some divide but mitoses are ordinarily extremely rare. There is a large numerical

opportunity for phagocytosis within, and especially without, the venous sinuses is excellent. Reticular cells, endothelial cells, and probably blood monocytes enlarge and produce macrophages. Many red blood cells are phagocytized.

The amount of blood entering the capillaries and venous sinuses is as in other situations regulated by the arterioles some of which have particularly thick walls. Circulation is so leisurely that it is facilitated by rhythmic contractions of the whole spleen made possible by the presence of smooth muscle in both the capsule and trabeculae. Obviously the spleen can greatly increase in size either rapidly by dilatation of the venous sinuses or slowly by proliferation of the lymphatic tissue.

*The thymus is an intimate mixture of scattered epithelial cells and of clumps (Hassall's corpuscles) with many lymphocytes in a background of reticular connective tissue.* It has no afferent lymphatic vessels, lymph sinuses or germ centers. It is much broken up into lobes and lobules. Both maximum development and regression are earlier than in other lymphatic organs. The thymus is not a filter for lymph. Its lymphocytes are not discharged in large numbers into efferent lymphatic vessels as in the case of subepithelial lymphatic tissue and lymph nodes. The function of the thymus still is unknown but it is more probably an expression of the dominating lymphatic component than of the recessive epithelial component.

## CHAPTER VIII

### ENDOCRINE SYSTEM

THIS system regulates bodily activities through the operation of a series of broadcasting stations, the tuning in of receivers and the resulting action. The endocrine glands are the stations. Each generates and emits a chemical substance or substances peculiar to it and not conflicting or drowning out those discharged by others. Like radio waves these hormones are all very penetrating. In the body medium, which is water, they easily pass through many obstacles. Produced by endocrine cells, they are first discharged into the immediately surrounding tissue fluid. Then they enter blood capillaries (and perhaps lymphatics), are widely distributed in the blood stream and eventually pass out through the capillary walls into the tissue fluids everywhere. But though all the cells in these fluid environments are exposed to them, in varying degrees of intimacy depending on vascularity, only those constructed as receivers for the particular substance respond by appropriate action. It is emphatically not an integration based on conduction along definite paths as in the nervous system, but one that depends on the building up of sufficient material for broadcasting effective concentrations in all directions.

**Thyroid.**—Like the thymus this organ develops from pharyngeal epithelium. It is not however shrouded in lymphocytes but maintains its epithelial character. Aristotle's designation, *globus hystericus*, tells part of the story because those who have hyperthyroidism are frequently geared up and nervous, while individuals suffering from hypothyroidism are subdued and apathetic. The broadcasted hormone, thyroxin, hastens one of the most universal of cellular activities in the body, namely *oxidation*, so that the receivers are legion. To make it *iodine* is one of the substances required and the amount of hormone manufactured is increased on receipt of *thyrotropic hormone* from the pituitary.

To bridge the gap between gross and microscopic appearance examine a human thyroid or the thyroid of a fairly large mammal. Observe its color and how it feels. Locate some of its vessels and nerves. Attempt to strip off its capsule. After thus exposing the surface examine pieces in water with a hand lens and identify the structural units known as *follicles* (Fig. 73). (The extraglandular lymphatic plexus will probably not be visible unless it has been exposed to India ink, or other material, which easily enters it after injection into the tissue fluid. At a slightly higher magnification the capsule, follicles, lymphatics and blood vessels are illustrated in figure 74.) In thick sections, cut with a razor blade, tease out a few individual follicles. In addition follicles isolated by maceration, should be studied. Jackson (1931) has standardized the procedure. Place pieces of the gland in 3 parts of concentrated hydrochloric acid and 1 part of water for twenty-four hours. Wash thoroughly in at least 10 changes of water and gently separate (Technique, p. 191).

Seen in ordinary hematoxylin and eosin stained sections the outstanding characteristics of the thyroid gland are readily made out. It is a mass of follicles (*L. folliculus*, diminutive of *folles* a bellows), closely knit together by connective tissue and serviced by blood vessels, nerves and lymphatics. The wall of each *follicle* is made up of a single layer of epithelial cells which betrays its surface origin. These cells are all of the same kind but show some variability because they are obviously not all of the same age. To make good occasional loss of cells by death, some divide but mitoses are ordinarily extremely rare. There is a large numerical

margin of safety. Follicular epithelial cells can pass their hormone directly into the surrounding tissue fluid or store it in the colloid substance within the follicle.

This colloid in stained sections often presents an irregular outline separated from the epithelium by a clear space (Fig. 78). Both outline and space are artefacts. In the living condition the lumen is completely filled. The colloid is chiefly acidophilic but it may exhibit diffuse or patchy basophilia. That this difference in staining is not entirely due to variations in technique is clear. The basophilic masses may



FIG. 73.—Camera lucida drawing of the surface or the extraglandular lymphatic plexus of a dog's thyroid showing individual follicles of thyroid and the communications from the interfollicular plexus running into the large lacuna of the surface plexus.  $\times 155$  (Rienhoff Arch. Surg.)

exhibit a bright blue autofluorescence (Grafflin 1939, 1940). According to Leblond (1943) in the relatively inactive thyroids of hypophysectomized rats the colloid is acidophilic while in the one activated by pituitary hormone it is mostly basophilic. In the colloid droplet of non-staining material may occur likewise a few degenerate epithelial cells.

Both follicular epithelium and colloid show striking changes in different functional states, many of which cannot be given without reference to *gouters*

the commonest cause of which is long standing iodine deficiency. In those of the colloid variety the gland is greatly enlarged and, on microscopic examination, the follicles are found to be distended with large amounts of colloid and the epithelium to be thinned out because the cells are spread over larger areas. One can imagine that the cells are attempting to do their duty, that is to make enough hormone for bodily needs, but that with an insufficient supply of iodine this results in the pro-

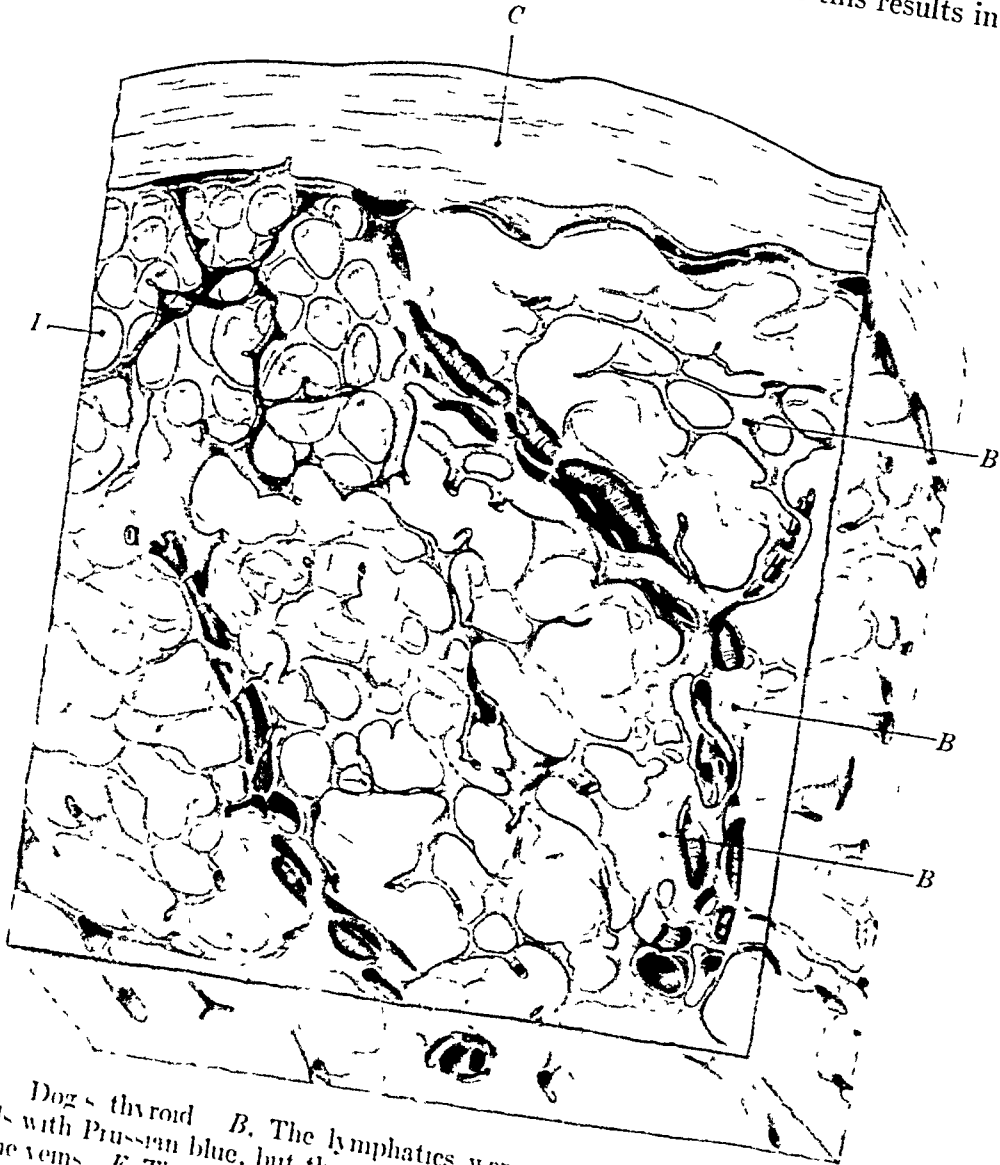


FIG. 71 Dog's thyroid. *B*, The lymphatics were injected with India ink and the blood vessels with Prussian blue, but the arteries are illustrated in red for the sake of contrast with the veins. *E*, The follicles, and *C*, the capsule, are shown. The tissue was cleared by the Spalteholz method and a thick free-hand section drawn with camera lucida.  $\times 57$  (Rienhoff, Arch. Surg.)

duction of enormous quantities of low grade colloid, certainly poor in iodine and probably also in hormone. The main direction of discharge is into the lumen where the colloid and the contained hormone is stored. As needed some of both pass back through the follicular epithelium into the tissue fluid. What forces bring about movement in this reverse direction are not known, but, if in this kind of goiter



remains of the ultimobranchial bodies may occur. In rats these can be the source of cysts, tumors and other irregularities (Van Dyke 1944). But the method is one of wide application because the distribution of other radioactive materials can likewise be determined in other tissues. A concise summary by Ross (1943) is recommended.

De Robertis (1942) has investigated the response of the follicular cells of guinea pigs to the thyrotropic hormone of the pituitary. Figure 76 demonstrates the promptness of the response. In the first phase the cells produce a large quantity of intracellular colloid which appears to be secreted into the follicular lumen. The cytoplasm of the cells stretches out into the lumen and is pinched off with the

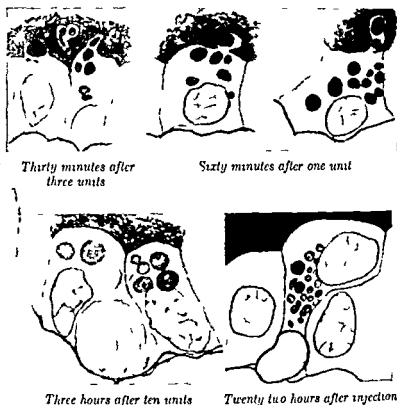


FIG. 76—Initial stages of thyroid activation in rats after giving thyrotropic factor of pituitary as seen in frozen-dried-denatured sections stained with aniline blue-orange (From de Robertis, courtesy of Anat. Rec.)

continued colloid droplets. In this case at any rate the colloid does not escape through the cell membrane; it merges with the colloid in the lumen where the detached investing cell membrane breaks down. A similar mode of exit of secretion obtains in the pancreas (Fig. 144). In the second phase the colloid seems to be secreted in the opposite direction toward the base of the cell and reabsorption of follicular colloid takes place.

In two excellent papers Williams (1939, 1941) has described the structural changes in living thyroid follicles of rabbits. He made thyroid grafts in specially constructed chambers and transilluminated the thyroid *in situ* by inserting a light into the trachea. In the phase of secretion the distal border of the cells next the lumen becomes indistinct and cytoplasmic processes extend into the lumen. This is

always associated with a relatively sudden and considerable increase in the amount of colloid. Williams directly observed the accelerating influence of pituitary hormone and the retarding effect of sodium iodide. He also noted some changes resulting from alterations in temperature which call to mind Kenyon's (1933) earlier demonstration of how profoundly the structure of the thyroid can be modified in the direction of hyperactivity by maintenance at low temperature (Fig. 77). The thyroid is evidently a unique and highly responsive assemblage of units which are epithelial sacs. The cells either secrete a colloid material charged with hormone into blind lumina where it is stored and whence it must make its way back through the epithelial walls before it can enter the surrounding tissue fluid or discharge it directly into the tissue fluid depending on circumstances. The gland

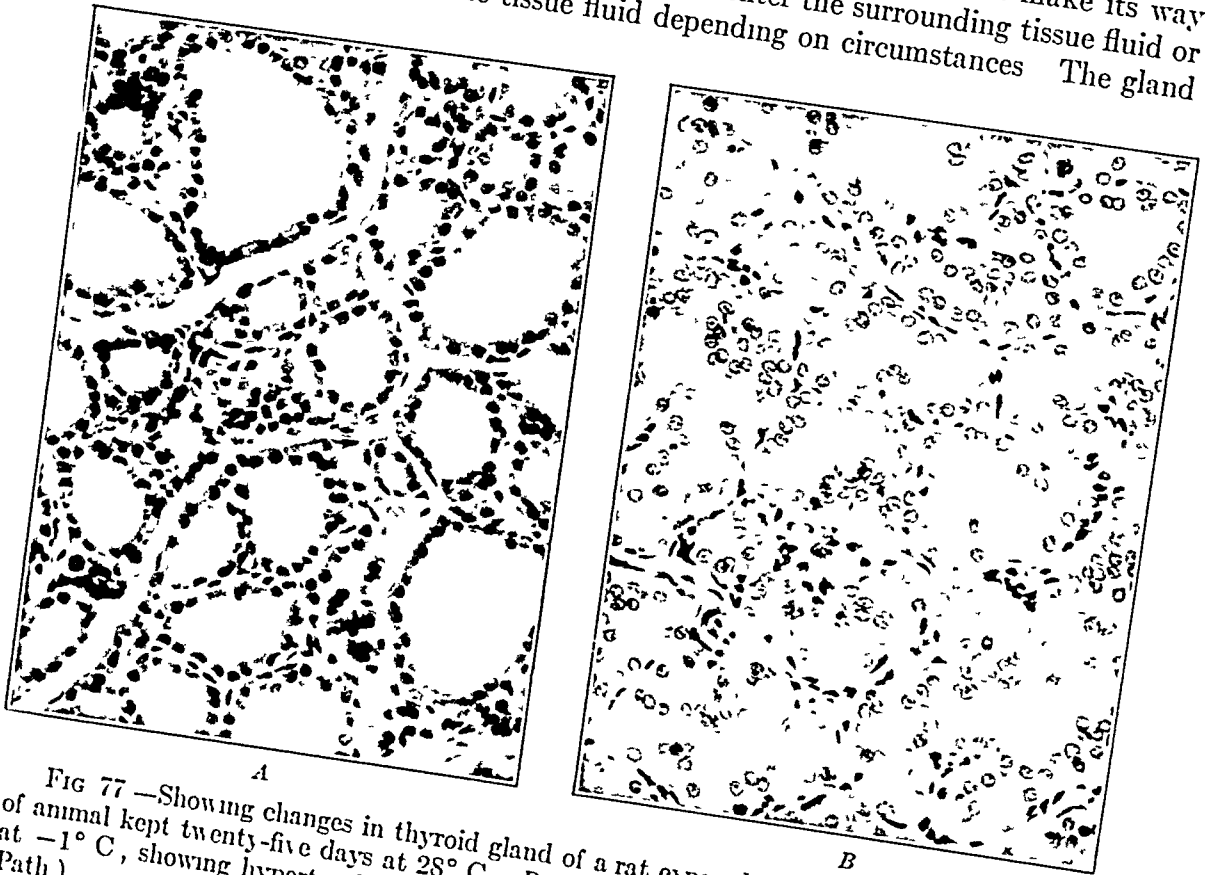


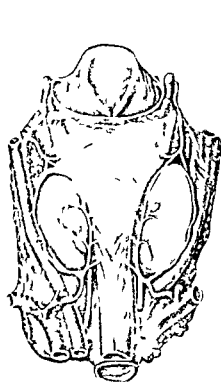
FIG 77 —Showing changes in thyroid gland of a rat exposed to cold. A, Normal gland of animal kept twenty-five days at 28° C. B, Gland of litter-mate kept twenty-five days at -1° C, showing hypertrophy of cells and loss of colloid.  $\times 275$  (Kenyon, Am J. Path.)

continues in service as long as life lasts. The thyroids of old people look surprisingly healthy (Fig 78), but they do age (see Carlson, 1942).  
**Parathyroids.**—These occupy a position close to the thyroid (*G para* beside) as illustrated in figure 79. They produce parathormone which helps in some way to regulate the level of blood calcium.

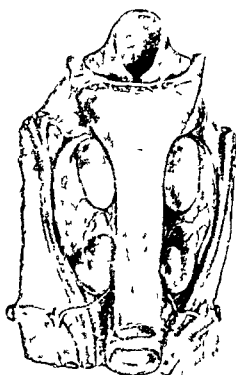
In this book we are concerned with the microscopic localization of chemical processes. We would like to know where are the receivers of this broadcasted hormone. According to Albright (1941) "the hormone affects phosphates in the circulating body fluids in such a way that their excretion in the urine is increased." This would, he thinks, increase the "tendency for calcium phosphate to enter the serum from the gastro-intestinal tract or from bone." On this theory, or any other



FIG. 78—Thyroid of a woman aged one hundred and eleven years  $\times 300$



*Normal parathyroid*



*Enlarged parathyroid*

11. (1) External view of thyroid and parathyroid glands. First in the normal state (H. B. Cooper, *Operative Surgery of Coiter*, Johns Hopkins Press) and second showing parathyroid enlargement (Stelling *The Parathyroids* St. Louis: Mosby 1935).

in which the hormone is supposed to act on some salt extracellularly placed in tissue fluid or plasma, the hormonal broadcasting is evidently not picked up by specially tuned cellular receivers. But it is possible that some of the hormone does act on particular cells and in fact stimulates local cellular action in bone whereby the matrix and contained bone salt are simultaneously dissolved as suggested by McLean and Bloom (1941) who make reference to the earlier studies by Shipley and Macklin and by Selye. The influence of this hormone on teeth is very noticeable (Fig. 104)

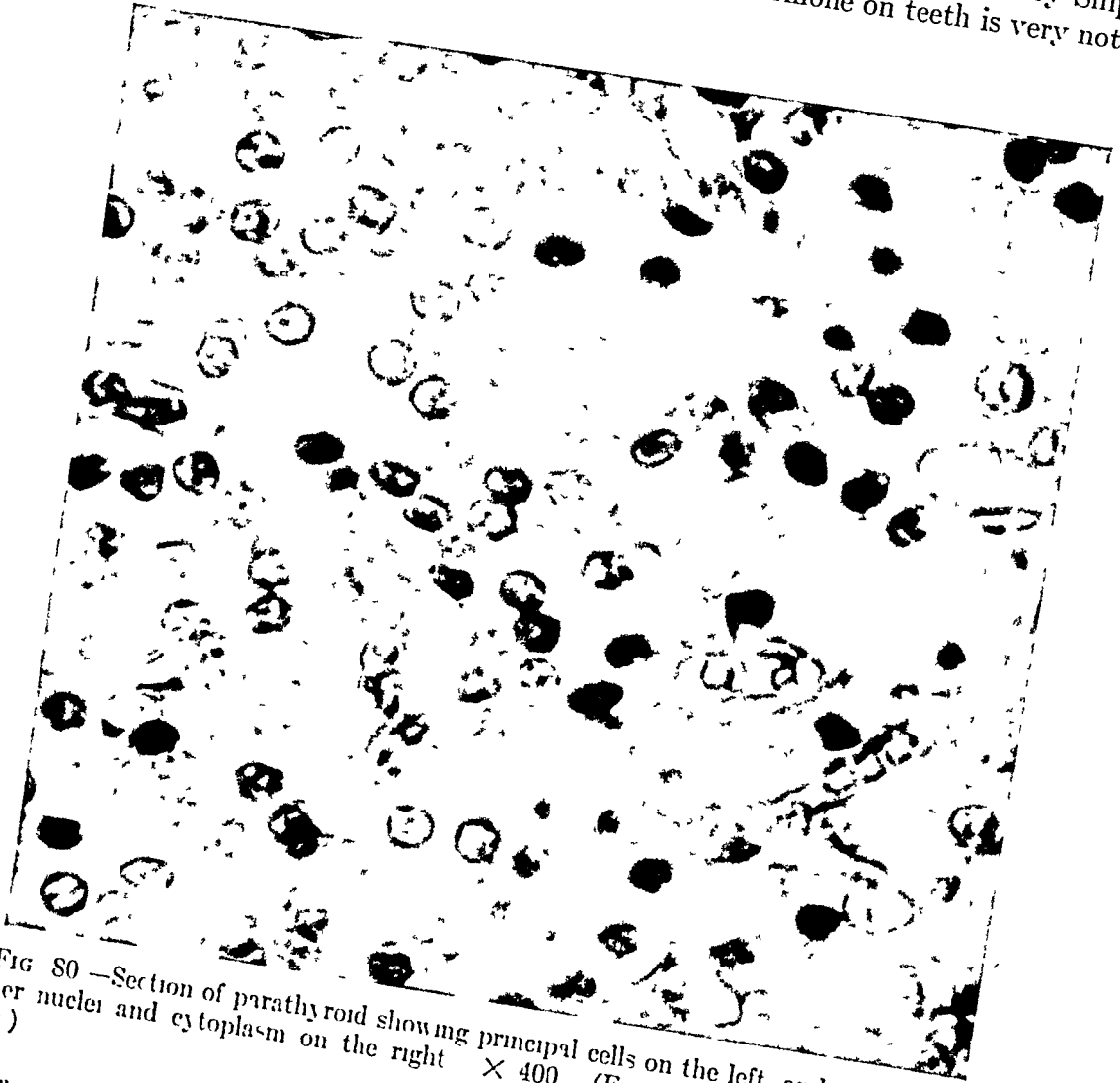


FIG 80 —Section of parathyroid showing principal cells on the left, and oxyphil cells with darker nuclei and cytoplasm on the right  $\times 400$  (From Morgan, courtesy of Arch Path)

The microscopic structure of parathyroids is refreshingly simple. Each gland is a small slightly elongated structure the smooth surface of which is limited by a delicate, connective tissue capsule. Its secretory cells, derived from pharyngeal endoderm, are clearly epithelial in nature. Their nuclei are larger and stain less intensely than those of lymphocytes. Their cytoplasm is about as extensive as that of thyroid cells. Neighboring cells are closely packed together with little intervening tissue fluid as is the habit of epithelial cells. Also, like epithelial cells, they are arranged in cords and occasionally in clumps about tiny roughly spherical lumina, which may contain a little colloid, but this colloid is said to differ from that

of the thyroid in having no iodine. Certainly there is no complicating storage phase as in the thyroid. But since these epithelial cells of the parathyroid have an important hormone to discharge they are not present in large, thick aggregates. On the contrary their distance from blood vessels (and the larger amount of tissue fluid about them) is never such as greatly to impede the flow of hormone.

The secretory epithelial cells are divisible into several ill-defined categories about which there has been much discussion. Even in papers by Castleman and Mallory (1931, 1937) and by Morgan (1936), covering work done in the same laboratory, difference of opinion is expressed. It is safe to recognize two main types.

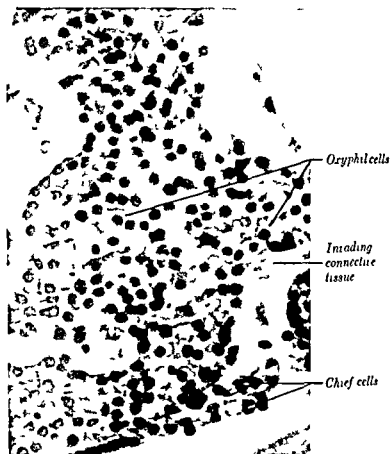


FIG. 81. Parathyroid of woman, aged one hundred and eleven years.  $\times 200$

**A Chief or Principal Cells** — These are the only cells found in young children. They are illustrated in the left half of figure 80. Their cytoplasm stains but faintly. Morgan describes two varieties — pale principal cells and dark principal cells. The former are probably the "water clear" (Wasserhelle) cells of Castleman and Mallory and the latter the normal chief or principal cells of these authors.

**B Oxyphil or Colloid Cells** — These usually appear in older children approaching puberty. They are illustrated in figures 80 and 81 and are normally greatly in the minority. Their cytoplasm colors with eosin and appears dark and their nuclei are rounded and very intensely stained. The same names are applied to certain cells in the thyroid gland, the cytoplasm of which is acidophilic (or oxyphilic) which are not found in youth and which are seldom very numerous. Both

Castleman and Mallory and Morgan report pale and dark oxyphiles, the difference, however, is one of degree and not of kind

These two cell types, with their varieties are to be regarded as stages in the life cycle of a single kind of secretory cell. The clear ones precede the oxyphil ones. From their study of tumors Castleman and Mallory conclude that the chief cells are the fundamental type always present, if even in small numbers, and probably the only phase in the life cycle which is proliferative. The oxyphil cells, particularly the dark ones, are senile

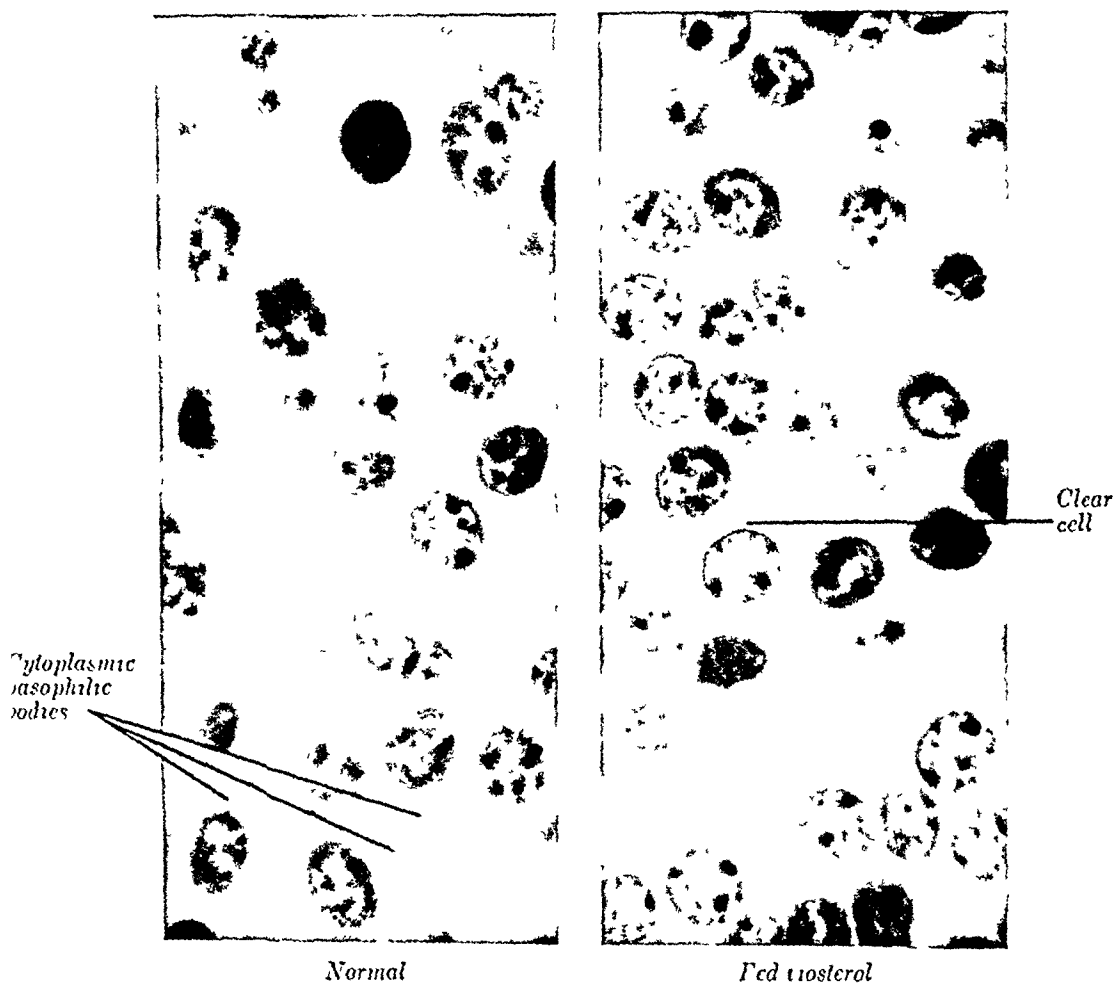


FIG. 82 —Comparison of normal parathyroid with parathyroid of monkey fed viosterol

Insufficient parathyroid secretion is seldom met with clinically. However, occasional tetany during pregnancy, is attributed by Marine (1932) to under-secretion. Over-secretion, or hyperparathyroidism, is now well recognized as a clinical entity (Barr and Bulger, 1930). It is accompanied by bony changes but whether these are produced by excess of hormone acting on living cellular receivers, or on salts in body fluids, or on both remains to be determined. When parathyroid cells become malignant those that invade other parts of the body are capable of exerting the same influence on calcium metabolism but they do so without restraint (Gentile, *et al*, 1941). The parathyroids are tremendously enlarged in renal rickets (Fig 79). A discussion of their physiological hypertrophy by Ham *et al*, (1940) afford welcome orientation.

De Robertis (1941) has investigated low calcium and low phosphorus rickets in rats. He found in both an increase in size of the gland, an increase in number of epithelial cells and cytological indications of activation of the cells but the hypertrophy was more marked in the low calcium than in low phosphorus rickets. Apparently the secretory cells make and discharge more hormone in an effort to restore the serum calcium and phosphorus to their proper levels. When monkeys are given vitamin D which increases serum calcium the demand for hormone is reduced and the structure of the parathyroids is altered by the disappearance of basophilic deposits (juxta nuclear bodies) in the cytoplasm (Cowdry and Scott 1936). See figure 82. There are several reports in the literature of depression in secretory activity following administration of parathormone which is what one would expect. Without such self regulation this and other glands would run wild in secretory activity making much more than is needed. Some believe that the pituitary sup-

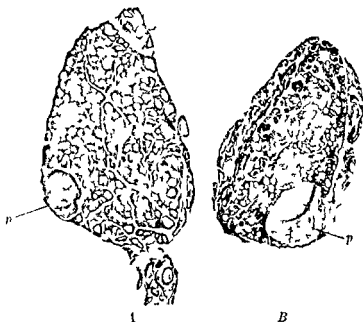


FIG. 83.—Effect of underfeeding on the thyroid and parathyroid glands (p) of the white rat. A normal B underfed. Size of parathyroid increased. (Jackson *Am J Anat*)

plies a special hormone that activates the parathyroids. But the influence of removal of the pituitary and consequently of any such hormone on the parathyroids is far from uniform. In some animals there is no resultant parathyroid atrophy. In the monkey Baker (1942) observed possible slight atrophy unaccompanied by significant structural alterations in the parathyroid cells. Innervation is not well developed and probably chiefly confined to the blood vessels. There is no evidence of the nervous control of secretion.

No technical method has been discovered whereby the hormone can be visualized within the secretory cells even in stages of hyperactivity. It is evidently produced in rather low concentration by a great many cells. The factor of safety in number of cells is therefore considerable. Compensatory hypertrophy (regeneration) following partial parathyroidectomy, was not noted by Rosol (1931). Yet in underfed young rats Jackson (1916) observed that the parathyroids are to be grouped with a few other organs whose growth energy is so great that they continue

to enlarge (Fig. 83). In extreme old age they exhibit some signs of epithelial atrophy and connective tissue increase (Fig. 81) After death they are peculiarly resistant to autolysis.

**Adrenals.**—The thyroid and parathyroids are single-duty endocrines; but the adrenals are double-duty ones, for, in them, two endocrine tissues are combined whose origin, structure and function are quite different

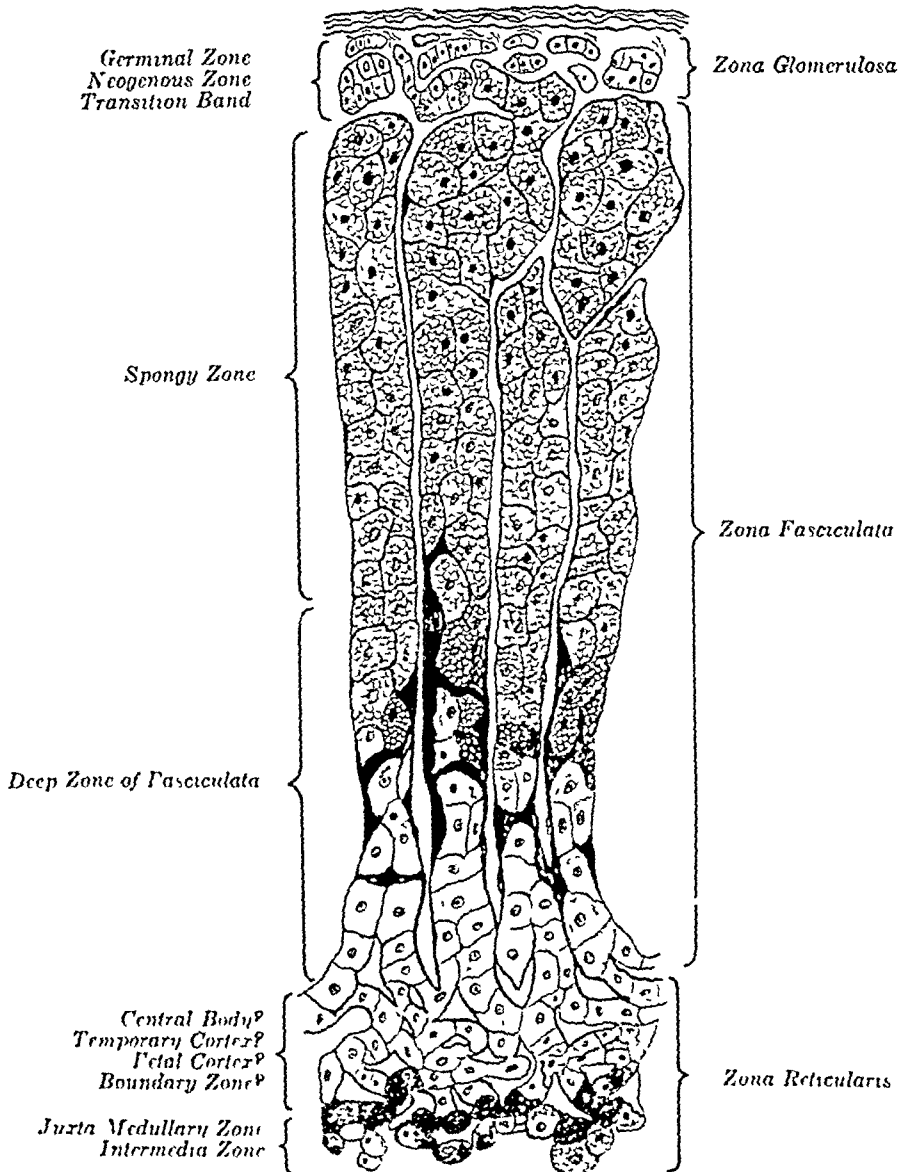


FIG. 81.—Section through the adrenal cortex (Redrawn from Goormaghtigh, 1922, *Le cortex surrénal humain dans les plans de l'abdomen et aux périodes intéressantes de la vie sexuelle*, Thèse, Université de Gand)

Distinction between adrenal cortex and medulla is fundamental. When a free hand section through a fresh adrenal is examined it will be seen that the cortex is of a pale yellow color, owing to its richness in fatty substances; while the medulla is darker, because of the relative absence of these substances and the presence of more blood. Application of a dilute solution of chromic acid slowly brings about a brown coloration of the medulla. This is the chromaffin reaction for epinephrine-secreting tissue.



The cortex is so constructed that very close association obtains between epithelium like cells of mesodermal origin and the blood stream. Through most of its thickness the cells are arranged in cords, or columns, seldom more than two cells in width which stretch from the capsule toward the medulla. Between these columns of cells are blood capillaries and a little reticular connective tissue. Arterial blood enters the cortex from vessels in the capsule, perfuses the entire cortex in these



*Intact normal female*



*Castrated female dust-like particles lost*



*Castrated male 100 mg hormone  
hypertrophy of cortex and loss of  
dust-like particles*



*Castrated male 500 mg hormone  
hypertrophy of cortex and storage of  
particles*

FIG. 8. — Shows influence of pituitary adrenotropic hormone on birefringent material in adrenal cortex of mice. (45) (Weaver and Nelson, courtesy of Anat. Rec.)

evenly pass capillaries and in an occasional arteriole and passes on to the medulla within. Thus there is established a vascular gradient, the outer cells are first served with arterial blood and the inner cells last. The flowing blood is progressively altered by what it gives up to the cells and by what it receives from them in return. Conditions of cell life likewise change. The cells live differently and live differently depending upon their position in the gradient. Those similarly placed

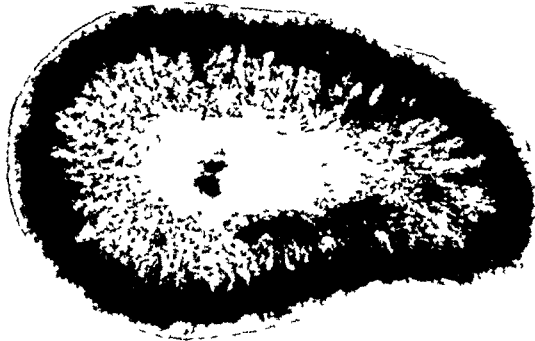
exhibit common properties and exist in zones roughly parallel to the capsule. A fundamentally similar cellular stratification exists in many other tissues. In the adrenal three zones are easily recognizable. These have been given many names. The acceptable ones are listed on the right in figure 84.



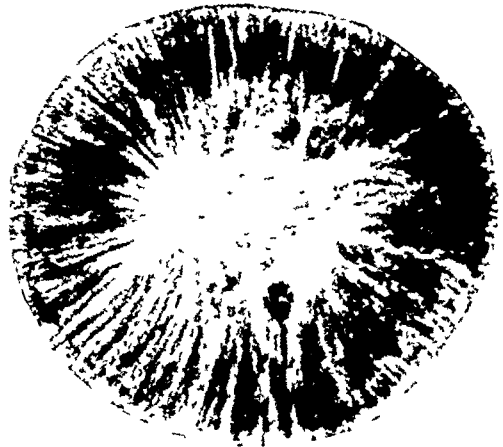
*Female less than 1 day old*  $\times 35$ .



*Female 87 days old*  $\times 16$ .



*Male 87 days old*  $\times 19$



*Male over 1 year old.*  $\times 10$ .



*Male 87 days old*  $\times 18$



*Male over 1 year old*  $\times 8$

FIG 86.—Variations in amount of lipoid in the adrenal cortex of normal rabbits. Transverse frozen sections colored with Sudan IV and hæmalum. The lipoid shows black or dark gray in the photomicrographs. The medulla is comparatively free of lipoid. (Slightly modified from Whitehead, courtesy of Jour. Anat.)

In the *Zona Glomerulosa* (dim. of *L. Glomus*, a ball) the pattern of vertically penetrating capillaries is being organized but the cells are still grouped in clusters. They receive arterial blood first. Hoerr (1931) has found that in the guinea pig the principal site of cell division is at the border between this zone and the underlying fasciculate. Interesting in this connection is evidence that in rats both the sudan stainable fat (Tobin and Whitehead, 1912) and birefringent lipoid material

(Wever and Nelson, 1943) are absent or less in amount at approximately this level than in the outer glomerulosa or in the deeper fasciculata (see the first photomicrographs of figure S5). Much cytoplasmic fat is a hindrance to cellular multiplication but does not actually prevent it except perhaps in fat cells (p. 278).

The *Zona Fasciculata* (d of I *fascis* a bundle) is much wider. The columns are more conspicuous and cells are larger and more heavily charged with lipoids and neutral fats. Not infrequently a gradation in properties of cells is noticeable in the fasciculata as one approaches the reticularis. The cells near the reticularis are apt to have less fat and to show more signs of ageing. The cell types sometimes

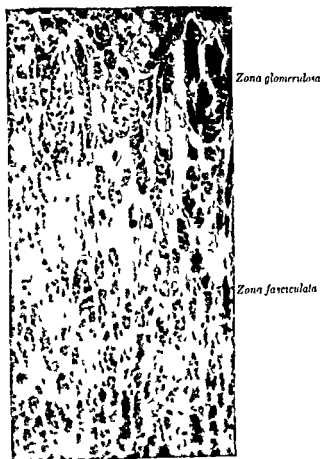


FIG. S7 - Senile atrophy in adrenal cortex of woman of one-hundred and eleven years  
 $\times 240$

described are undoubtedly stages in the life cycle of a single kind. The fasciculata gradually merges with the reticularis.

In the *Zona Reticularis* the close relation of cells to capillaries is maintained but the capillaries are no longer parallel. They form a kind of a network and the cells likewise. There is less fat in the cells than in the fasciculata but considerable pigment is present especially in elderly people. Signs of cell death are more noticeable.

The cortex undergoes marked alterations in size. There is extensive hypertrophy during fetal development, puberty and pregnancy and in scurvy. Marked enlargement follows castration, cholesterol feeding and administration of pituitary-adrenal atrophy hormone. There is atrophy in Addison's disease.

Hartman (1942) has concluded a review of the functions of the adrenal cortex with the statement that it "plays an indispensable rôle in practically all activities of the organism." What however are its primary duties? The redistribution of water throughout the body, mentioned by Hartman as a consequence of adrenalectomy, is basic. An increase in permeability of capillary endothelial walls takes place, tissue fluid increases and the cells in the fluid absorb water. How much this sweeping change is due to impairment of the ability of the kidneys to retain sodium chloride and to remove potassium remains to be determined. Our task as histologists is to try microscopically to localize significant chemical changes within the cortex. Marked variations encountered in the size of the 3 zones are well illustrated by Zwemer (1936).

1 Vitamin C (ascorbic acid) is certainly stored within the fasciculata and reticularis, apparently not in the glomerulosa (see illustrations of Giroud and Leblond, 1934). Cellular activity in this connection is described by Bourne (1942).

2 Lipoids, mostly birefringent cholesterol and cholesterol esters, are stored chiefly in the fasciculata. Knouff *et al* (1941) found that excessive muscular activity causes a reduction in cholesterol. Correlated histological and chemical studies of adrenal lipids by these authors are valuable. Dosne and Dalton (1941) think that decrease in lipid and cortical hypertrophy are indicative of increased hormone production.

3 Cortical hormones (cortins or corticosterones) are several in number. Weaver and Nelson (1943) have reported remarkable changes in the amount of birefringent lipid material caused by administration of adrenaltropic hormone of the pituitary as seen in polarized light (Fig 85). The presence of dust-like particles within the walls and lumina of the capillaries is regarded by them as evidence of the release of cortical hormone.

4 Some male and female sex hormones are closely related chemically to the cortical hormones. Together they comprise the group of steroid hormones. The female one, progesterone (p 324), has been isolated from cortical extracts, and cortical tumors may result in clinical manifestations very similar to ovarian tumors. Salt and water retaining properties of progesterone and testosterone recall those of corticosterone. The length of survival of adrenalectomized rats is increased by progesterone (Bourne, 1939). There is reason to suspect that the zone of cortex next to the medulla (see X Zone, McPhail and Read, 1942) is most directly involved with sex hormone. Grollman (1936) has dubbed this zone of mice the "androgenic zone."

5 As the blood is forced through these penetrating capillaries along the sides of columns of fat and lipid laden cells, it is subjected to a kind of filtration. Detoxification of the blood stream is claimed by some. Among the substances obviously picked up are particulate materials. The endothelial cells of the reticularis are energetic phagocytizers and thus qualify for listing as components of the reticulo-endothelial system with other "special endothelial cells." It is the reticularis, also, that shows the earliest lesions when the composition of the blood is unfavorable in fevers, acute infections and other conditions.

Alterations in level of the various zones with changing demands upon them are likely to occur. The entire cortex is to be regarded as endowed with potentialities for carrying out the functions mentioned, regeneration, hormone production, vitamin C storage, etc. Superficially it bears a striking resemblance to hepatic tissue (p. 190).

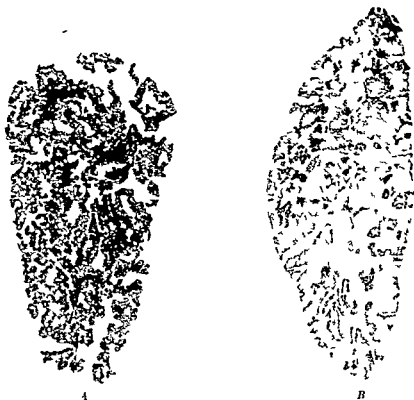


FIG. 88—Photomicrographs of adrenal medulla (of rats) fixed in formol Muller and exhibiting the chromaffin reaction of browning of cytoplasmic granules here represented as a blackening. *A*, The normal and *B*, exercised at room temperature to the point where body temperature fell from  $35.8$  to  $33.8^{\circ}\text{C}$ . (Redrawn and modified from Vincent Quart. J. Exper. Physiol.)



FIG. 89—Discharge of adrenalin: a. apnoea. Mouse killed by breathing atmosphere rich in carbon dioxide. Note the granules of adrenalin blackened by the treatment with osmic acid in the medullary cell and venous spaces.  $\times 550$  (Cramer Fever Heat Regulation Climate and the Thyroid and Adrenal Apparatus Longmans, Green & Co. Ltd.)

The adrenal *medulla* is evidently shielded by the cortex. In consequence of this arrangement, which has gradually come about in the evolution of vertebrates, most of the blood that reaches the medulla is certainly more venous and less arterial than it would have been had it not been filtered through the cortex. Harmful substances in it may have been partly or completely removed. Perhaps others useful to the medulla have been added. Certainly this blood is especially rich in corticosterones and may possess much vitamin C liberated from storage. The medulla is more conservative and does not undergo such great changes in volume as the cortex.

The capillaries in the medulla anastomose and run in all directions. They are wider than in the cortex, can properly be termed sinusoids, and are drained by thin walled venules. The medullary cells are of ectodermal origin and give a pronounced chromaffin reaction. In the course of development unknown forces have caused them to occupy this central position. Never is it the other way about with chromaffin cells peripheral and lipid laden cells central. But some chromaffin cells occur in small clumps retroperitoneally (paraganglia) not associated with cortical tissue.

Only one hormone is made by the medullary cells, epinephrine, of which the trade name is adrenalin. Tiny granules, demonstrable by the chromaffin reaction within the cells, are its precursor. A better reaction is probably that of blackening with osmic acid. The number of granules both in the cells and in the blood can easily be altered experimentally (Figs 88 and 89). Cytological manifestations of secretion are described by Bennett (1941) whose fine pictures should be examined. Medullary tumors are associated with paroxysmal hypertension due to the discharge of too much epinephrine. Numerous nerve fibers enter the medulla through the hilus. Most of these end in touch with the chromaffin cells but a few pass out into the zona reticularis of the cortex. Lymphatic capillaries are not well developed. Some begin in association with the veins of the medulla and become confluent forming vessels that leave with the veins. But vascular drainage of tissue fluid from the cortex appears to be so proficient that lymphatic capillaries are not called for. All blood leaves at the hilus by a single adrenal vein in the wall of which smooth muscle is distributed in longitudinal bands. If this muscle were to contract rhythmically it might suck the blood out.

The adrenal as a whole contains about 80 per cent of water and is therefore one of the most watery organs in the body. This fact is to be remembered in connection with regulation by its cortex of water distribution.

**Pituitary.**—"Nature saw fit to enclose the central nervous system in a bony case lined by a tough, protecting membrane, and within the case she concealed a tiny organ which lies enveloped by an additional bony capsule and membrane like the nugget in the innermost of a series of Chinese boxes. No other single structure in the body is so doubly protected, so centrally placed, so well hidden. Her acts being purposeful, she must have had abundant reasons for this, and man's prying curiosity impels him to ask what they were" (Cushing, 1932).

Unless micro-copic study of the pituitary is related to gross study of the organ and its parts no clear idea can be obtained of this, the master endocrine. Demonstration of a median sagittal section through the entire brain and skull case showing the pituitary within the sella turcica and its attachment to the brain is helpful to begin with. Then a brain, removed with pituitary attached should be examined. Finally median sagittal sections of the pituitary, its stalk and the floor of the third ventricle should be studied at low magnification.

The pituitary in common with the adrenal is an association of two different tissues. The *pars buccalis* is a growth upward of a pouch of oral ectoderm while the *pars nervosa* is a projection downward of the tissue in the floor of the third ventricle also ectodermal. Viewed in a median sagittal section the four divisions of the pituitary are easily defined

<i>pars buccalis</i>	{	<i>pars tuberalis</i>	{	<i>pars distalis</i> —anterior lobe	{	<i>pars intermedia</i>	{	posterior lobe ( <i>neurohypophysis</i> )
(adenohypophysis)								
<i>pars nervosa</i>								

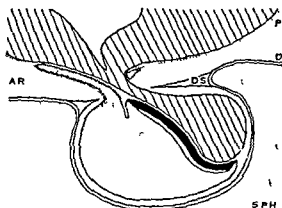


FIG. 90.—Diagrammatic sagittal section of pituitary illustrating relation to meninges. Brain floor and *pars nervosa* are lined; *pars distalis* lightly stippled; *pars tuberalis*, closely stippled; *pars intermedia*, solid black. AR, Arachnoid spaces; D, dura; DS, diaphragma sellae; F, fornix; SPH, sphenoid bone. (Atwell, *Am. J. Anat.*)

The *pars tuberalis* (heavily stippled in Fig. 90) extends upward toward the brain both in front and behind the infundibulum. It is inconstant of small size and need not be mentioned again.

The *pars distalis* (lightly stippled) forms the bulk of the organ. It is distal in the sense that it is remote from the brain in comparison with the *pars nervosa* which is itself a part of the brain. The *pars distalis* is also known as the *anterior lobe* because it comes before the *intermedia* from which it can readily be separated along the intraglandular cleft. This cleft is a vestige of the original lumen of the *pars buccalis*.

The *pars intermedia* (black) is developed from the posterior wall of the *pars buccalis*. It is adherent to the *pars nervosa*. Together these two form the posterior lobe. The *pars intermedia* is highly variable and in some species is not detectable. In man Risius (1925) found its weight to average only 0.0046 gm.

The *pars nervosa* (diagonal lines) is connected with the brain by the infundibulum (a funnel). It forms less of the pituitary than the diagram of a median sagittal plane through the organ suggests because the *pars distalis* is considerably wider.

From the structure of the *anterior lobe* one would not suspect that it produces more hormones than any other tissue (see Figs. 91 and 92). It is held together by a rather thin capsule. The secretory cells are epithelial since their forefathers had a lumen communicating with the outside world. Occasionally they themselves line up about small lumina. Such rounded formations are called acini (L. *acinus*, grape). Two are illustrated in figure 92. In general however, the secretory cells

are arranged in cords, or sheets, close to capillaries and embedded in reticular connective tissue. Never are they in clumps so thick, or dense, as to interfere with fluid exchange. Some of the capillaries are large and sinusoidal. These are lined by phagocytic endothelial cells which are components of the "special endothelium"

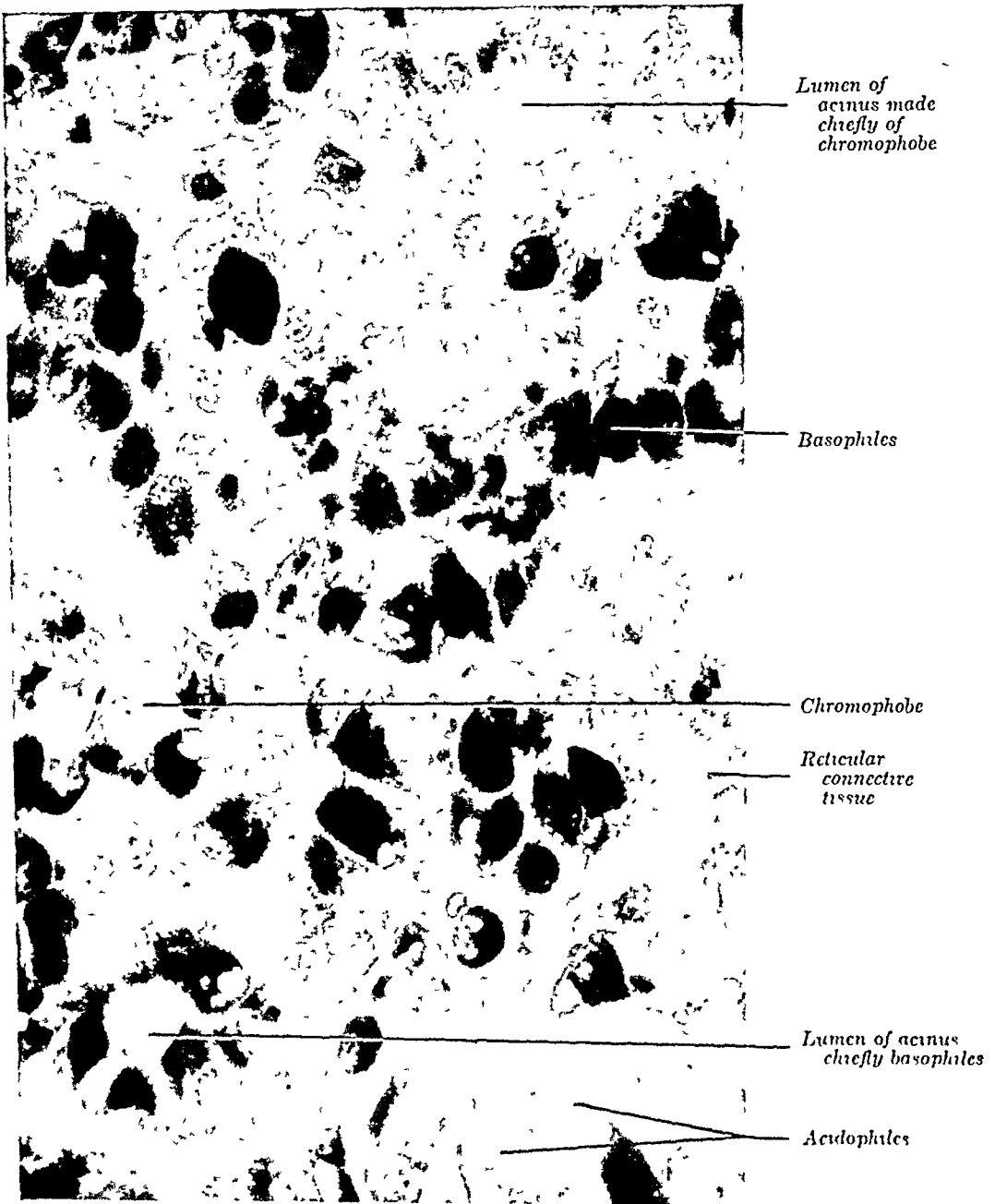


FIG. 91 - Pars distalis of fifteen year old white male who died with chorioepithelioma of pituitary. Ten per cent formalin, H & E  $\times 620$ . (Dept. of Pathology, Washington University, 19085, courtesy of Dr. R. E. Stowell)

of the reticulo-endothelial system. Here, as in the adrenal cortex, association of blood stream with secreting cells is unusually intimate.

Sections of the anterior lobe are easily recognizable by this arrangement of epithelial cells and by their grouping into 3 types.



1 *Chromophobes* are the most numerous (Rasmussen 1933) and usually the smallest. Their cytoplasm is chromophobic in comparison with the others so that they stain but lightly. They are non granular in the sense that they do not contain

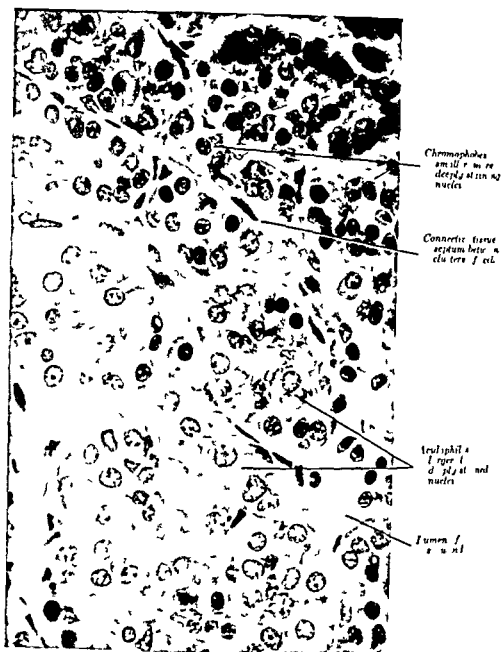


FIG. 92. Pars distalis of pituitary of fifty year old white male who died following pneumonectomy for cancer of bronchus. Regaud fixation H & E.  $\times 620$  (Dept. of Pathology Washington University, St. Louis, courtesy of Dr. R. L. Stowell.)

either of the specific granules characteristic of the other two types. They resemble the primitive cells of the embryonic pars distalis. Symptoms produced by tumors composed of chromophobic cells do not indicate the liberation of any physiologically active substance, but rather an insufficiency of hormone production caused perhaps

by pressure on the acidophiles and basophiles for the organ is lodged in a bony excavation which makes expansion difficult.

2 *Acidophiles* contain granules that stain with acid dyes. These are less numerous than the chromophobes and a little larger. Tumors of acidophile cells, are accompanied by overgrowth of a definite kind leading to acromegaly or gigantism. They are responsible for production of growth hormone and are said to manufacture adrenaltropic hormone as well.

3 *Basophiles* are least numerous and often the largest of the three. Tumors of basophile cells lead to overdevelopment of secondary sexual characteristics and there is other evidence pointing to the conclusion that they manufacture gonadotropic hormone.

This leaves us with the cellular origin of at least 4 other pituitary hormones (lactogenic, diabetogenic, thyrotropic and parathyrotropic) still to work out. It is not considered likely that they come from the chromophobes. Perhaps all of these 7 physiologically active substances may not represent the culmination of individual and distinct secretory acts. To express it differently, some may be necessary stages in the production of others, with end products and intermediate substances both capable of influencing vital processes. Recently attention has been directed to a trophic influence of the pars distalis on the kidneys (Heinbecker, Rolf and White, 1943).

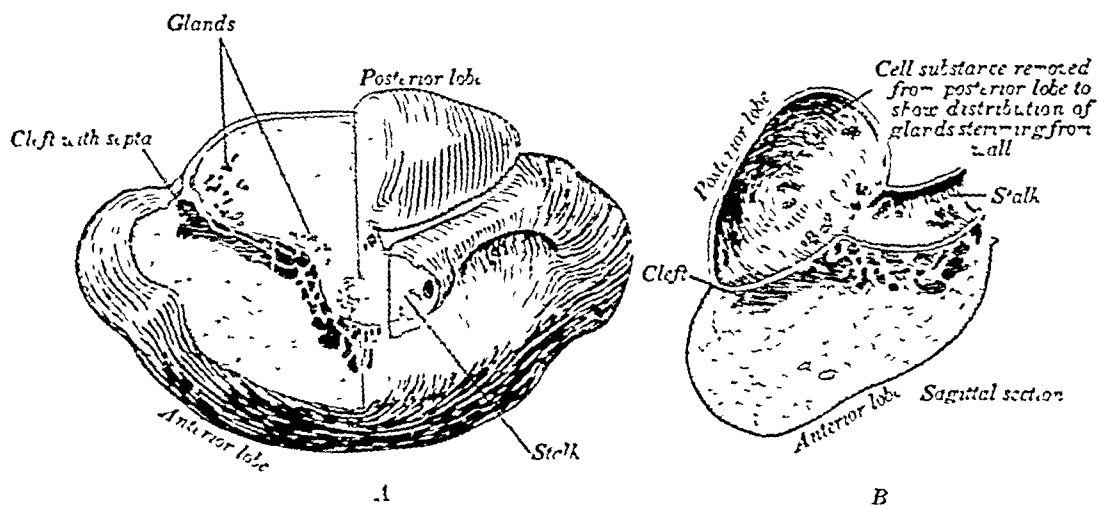


FIG 93—Pituitary of a child, aged one year. A, From behind with part removed, showing stalk and glands projecting from pars intermedia into posterior lobe (pars nervosa) B, Viewed in sagittal section with the substance of posterior lobe removed, leaving the invading glands.  $\times 5$  (Lewis and Lee, Bull. Johns Hopkins Hosp.)

But, because all the cells of a given type look alike does not preclude the possibility that some are making one product and some another. That the cells so seldom get out of hand is remarkable. The needed amounts of all the substances are usually produced in an orderly fashion throughout life. Neighboring cells of the three types live in practically the same tissue fluid. Their responsiveness to changes in this fluid is not the same. All broadcasted hormones enter the fluid and many other substances in addition. And, further, in a given cell it is possible that the responsiveness may be differential calling into activity one process and leaving another unawakened. Were it not for this difference in responsiveness cells inhabiting the same tissue fluid would have a strong tendency to become similar. But to

explain what conditions this differential responsiveness and why it persists is beyond us. We can only assume that the differences in reactivity are deeply ingrained in cellular organization. Another feature of these cells is the rarity of division under normal conditions. They are clearly long lived cells in which the specialization

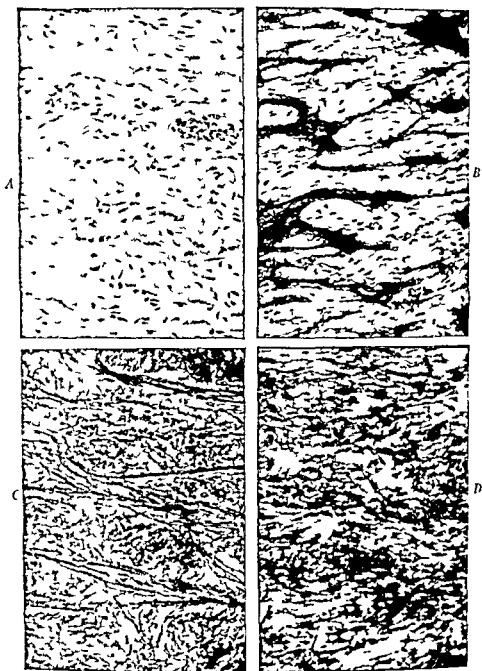


FIG. 4. Comparative study of the pars nervosa of the pituitary of the ox. A The usual laboratory stain gives little indication of the histological structure. Hematoxylin and eosin. B Connective tissue septa composed of fibers of reticulin and collagen. Iron-haematoxylin stain (Bischowsky's method). C Massive network of nerve fibers. Carazzi's silver pyridine technique. D Fibrocytes with their numerous processes. Note the varied structure. E. 111. Impregnation of Hortega's silver-carbonate method.  $\times 200$  (Bailey and Widry's Special Cytology, Paul B. Hoeber Inc.)

possible in terms of hereditary endowment is slowly attained and lasting. Smith and MacDowell (1930, 1931) have made the remarkable discovery that a defective gene leads to absence of acidophiles in mice in which the growth influence is lacking though the gonadotropic one operates.

The posterior lobe consists of the pars intermedia and the nervosa. The pars intermedia, more than any other portion of the pituitary, displays properties which seem to recall its origin as a diverticulum of the buccal ectoderm. This epithelium of the wall of Rathke's pouch in contact with the pars nervosa differs from that of the free or distal wall by: (1) lessened ability to grow and extreme variability in the size attained; (2) the exaggeration of colloid production, or failure of its elimination; (3) the occasional retention of primitive features such as ciliation and mucus formation, and (4) the suppression of the differentiation of acidophiles. The intermedia apparently lags behind the distalis in the variety of hormones produced. Acidophilic cells, so prominent in the distalis, are seldom, if ever, encountered; but the basophiles are fairly numerous. Excellent illustrations and many details are provided by Rasmussen (1930) and Lewis and Lee (1927). A series of transitions may be established between these cells and the less deeply staining chromophobes that line the several lumina. Fully differentiated basophiles occur side by side with chromophobes, and, as in the pars distalis, may contain a few colloid droplets; but the basophiles are also found singly and in clumps apart from the lumina. They reveal the remarkable property of invading the pars nervosa (Fig. 93).

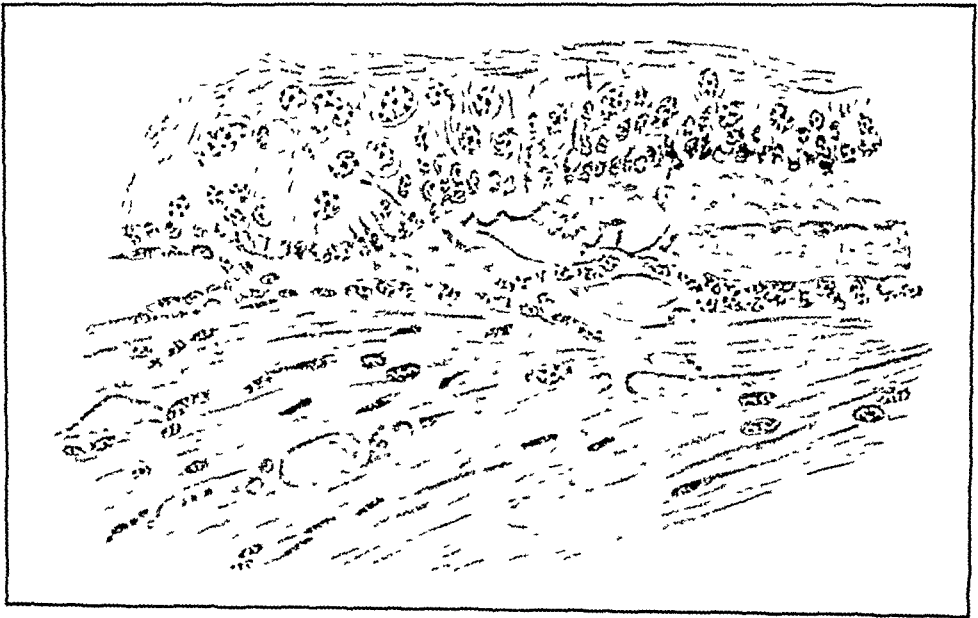


FIG. 95.—Section of pars nervosa of pituitary of cat, showing ependymal cells lining an extension of the infundibulum. The hyaline bodies of Herring are seen between the fibers of the nervosa, in the ependymal epithelium and in the lumen. (Redrawn from Herring, Quart. J. Exper. Physiol.)

Except for these basophiles the *pars nervosa* contains no cells of epithelial lineage. Nerve fibers sweep down into it from the brain and apparently end there. It is irregularly divided by connective tissue septa. Numerous cells, called pituicytes, occur (Fig. 94) but their nature is not known. Certain hyaline bodies are found in the tissue fluid mostly near the overhanging lumen of the infundibulum



FIG 96 —Pineal of five year old male. Groups of pineal cells separated by connective tissue. Small concretion below and to the right. Formalin fixation, H & E.  $\times 145$  (Specimen given by Dr W O Russell)

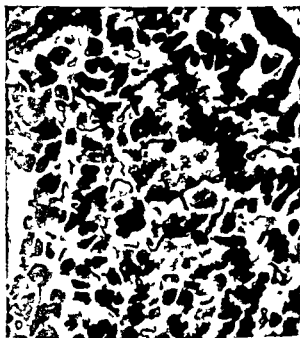


FIG 97 —Pineal of newborn male. Note bulbous, hook like and loop like processes of the pineal cell. Hirtaga's silver carbonate method for astrocytes.  $\times 650$  (Photomicrograph by Dr W O Russell)

(Fig 95). It has been claimed that they represent secretion being passed into the third ventricle but proof is lacking. Extracts of the posterior lobe contain active principles sold under the trade name of pituitrin. These produce a rise in blood pressure owing to vasoconstriction. They also bring about uterine contractions in labor and other results. Their production *in vivo* is difficult to measure and histological examination of the pars nervosa does not afford any clue either to their exact site of origin or to their volume of production as with some anterior lobe hormones.

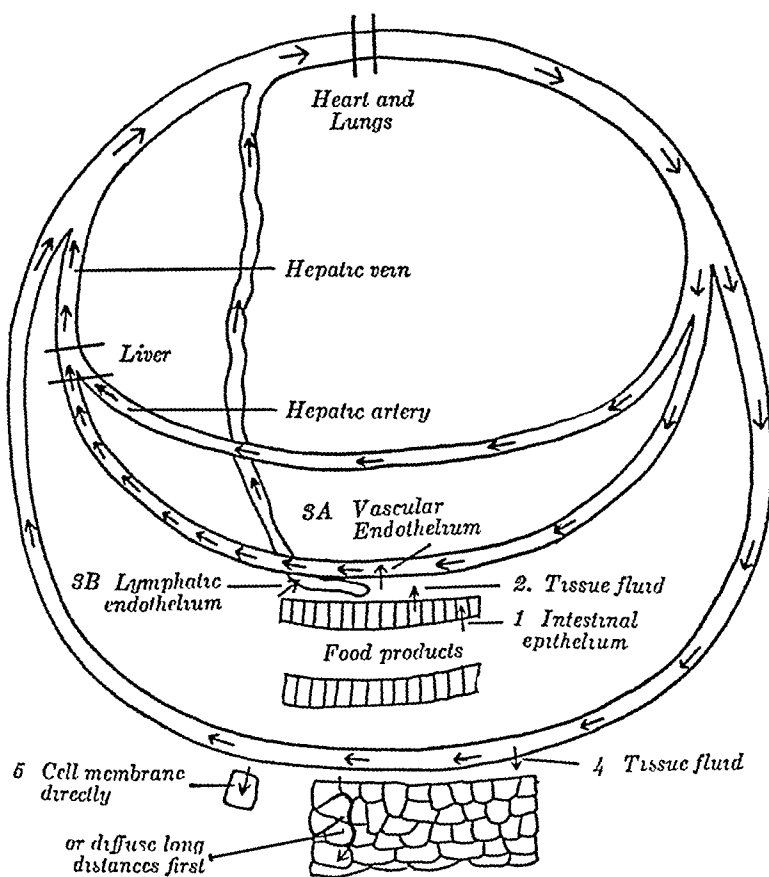


FIG 98.—Diagram of circulation of food products

**Pineal Body.**—This structure comes as a kind of antichiasm to the pituitary, the greatest of endocrines, because there is no longer any reason to consider it as an endocrine. Patients with pineal tumors are without symptoms indicative of endocrine disturbance (Russell and Sachs, 1943). Pinealectomy of three successive generations of parent rats produces no appreciable change in body size, rate of development or microscopic structure of endocrines (Sullens and Overholser, 1941). The pineal body of human beings is merely a vestige of the parietal eye of lower forms (Russell and Gregory, 1943). Yet one must be familiar with its appearance. The first point to be noticed is the presence of numerous cells arranged in indistinct clusters separated by strands of connective tissue. Attempts to discover a lumen and secretion antecedents within the cells are fruitless. Calcium deposits, that color blue with hematoxylin, are numerous near the blood vessels and in the cell clusters (Fig 96). Silver stains reveal remarkable loop and comma shaped nerve endings (Fig 97). The structure looks purposeful.

## SUMMARY

The thyroid parathyroids adrenals and pituitary are professional endocrines. That is in consequence of growth and differentiation they have become specialists and devote themselves entirely or almost entirely (adrenals), to integration of the body by broadcasting. Historically they are the aristocrats having served in leading capacities throughout the evolution of vertebrates. It is interesting that the thyrotropic pituitary hormone acts as a stimulant of the thyroid in mammals, birds and amphibia. This and other evidence leads Adams and Allen (1942) to write in favor of the zoological non specificity of vertebrate endocrines. The mechanism of cellular responsiveness once introduced by Nature has certainly shown little change. As a rule the producing cells are so constructed that they are immune to their own hormones. Thus thyroid extract does not stimulate the thyroid. A few hormones apparently act on extracellular chemical substances. The single structural requirement of all endocrines is close association of the secretory cells with the blood stream. In tumors this condition still holds for the cells must live. Consequently the cells of most endocrine tumors continue to manufacture and discharge their hormones but in an unregulated way *productive of excesses*.

Two other groups of endocrines will be described later. They are amateurs in the sense that the organs in question have other duties to perform. The sex hormones are produced in special parts of the testicle and ovary. The alimentary tract hormones comprise insulin manufactured in the pancreas an appendage of the tract and secretin and cholecystokinin produced by duodenal epithelium. The latter are interesting because the structural cellular organization required for their formation is so slight as to escape detection.

## CHAPTER IX

### UPPER ALIMENTARY TRACT

THIS book centers about the blood as the fundamental integrator of all vital activities. It is *via* the blood stream that the endocrines broadcast their messages. And it is from the blood stream, as a kind of revolving table, that the cells of the body feed. Figure 98 is intended to illustrate, very schematically, the sequence of events, indicated by the numerals 1 to 5. Food products moving in the blood vascular and lymphatic channels are indicated by black arrows and those passing through membranes by yellow arrows.

1. From the digestive tract they are chiefly absorbed by the epithelial cells of the small intestine

2. They enter the underlying tissue fluid, whence there are two paths of absorption:

3A. Most of them penetrate through an endothelial barrier into blood vessels and are wafted in the portal vein to the liver (*L. porta*, a gate). This vein is the gateway through which these adsorbed food products must pass. They are received by the liver which is a kind of guard by whom they are inspected, passed on quickly in the venous blood stream to the heart, or they are stored for subsequent use.

3B. Other food products enter the intestinal lymphatics by penetration through a barrier made up of lymphatic endothelium. These are poured into the venous blood shortly before it reaches the heart

4. Having been passed through the pulmonary circulation, substances taken in by both routes, are forced, in the arterial stream, to all parts of the body. They now move out through the vascular endothelium into the tissue fluid

5. From the tissue fluid they penetrate directly through cell membranes into nearby cells in vascularized tissue, or diffuse comparatively long distances in the tissue fluid to reach distant cells

The diet offered is very different from that of free living protozoa who are exposed to all materials in their immediate environment. The cells of our bodies have been pampered for millions of years. The substances that reach them in the tissue fluid have been carefully selected and predigested. Fatal results would now follow upon their exposure to an uncensored diet of raw materials. They are particularly susceptible to protein which has not been simplified by digestion.

We have seen that the food products in the intestinal lumen have to pass through an epithelial barrier, some tissue fluid and a layer of vascular or lymphatic endothelium before they can be carried around on the revolving table. The cells, to be fed, must behave with due decorum. They are not allowed to help themselves. They are indeed held away from the food by vascular endothelium. The majority have front seats, separated from the endothelium by only a little tissue fluid but some in avascular tissues (cornea, epidermis, cartilage, etc.) are placed much farther away.

Not all of the substances taken in food are harmless. Indeed the alimentary tract vies with the respiratory system for first place as the main portal of entry of disease provoking agents. Consequently its epithelial lining must give protection as well as absorption. Marked division of labor exists in the segments of the tract.



**Oral Cavity**—In this the first portion rejection is provided for by the testing substances through the sensation of taste in the tongue and widely distributed touch and pain nerve endings. When the signals are favorable the first part of the act of swallowing food ground up by the teeth and partly digested by admixture with secretions of the salivary glands is consciously initiated.

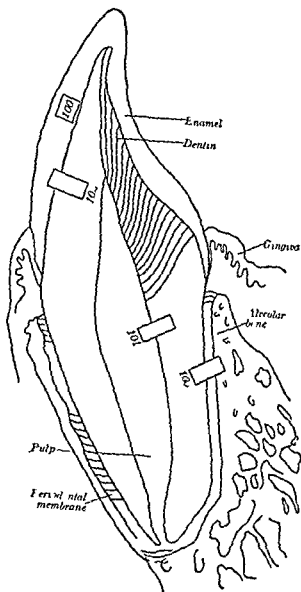


FIG 99 Tracing from a photograph of a decalcified section of an upper lateral incisor from a man aged twenty four years showing the location of the sections illustrated in figures 100 101 102 and 103 (From photograph by Dr Isaac Schour)

A prerequisite to the study of the tissues of the oral cavity is the examination of material from the cheeks the surfaces of the teeth the tongue and the tonsils. The materials should be collected as scrapings with a blunt instrument like the handle of a scalpel mounted on a slide and immediately covered with a cover glass without the addition of any physiological salt solution and examined first at low magnification and then with an oil-immersion lens. The student will observe epithelial cells mucus leucocytes and bacteria. He should study particularly the former for comparison later with the epidermal cells of the skin.

**Teeth.**—The roots of the teeth are lodged in the alveolar cavities of the jaws, where they are cushioned and held in place by the periodontal membranes, and receive the blood, lymphatic and nerve supply necessary for their maintenance (Fig. 99). Their crowns project into the oral cavity and are invested with enamel, the hardest substance produced in the body. Enamel in the dried state even sparks with steel like flint. It is the enamel which provides the cutting, crushing or grinding edge. Black (quoted from Alvarez, 1929) has found that the molars crush with a force of 100 to 160 pounds, maximum somewhere about 270 pounds; but the subjects on which the measurements were made hesitated to use their full strength because it made their teeth hurt. Thus, the enamel must withstand pressures to which no other parts of the body are regularly subjected. In gymnasts, supporting several men on their shoulders, the pressure on the feet is spread over an area far greater than the crowns of opposing molars in cracking a hard nut.

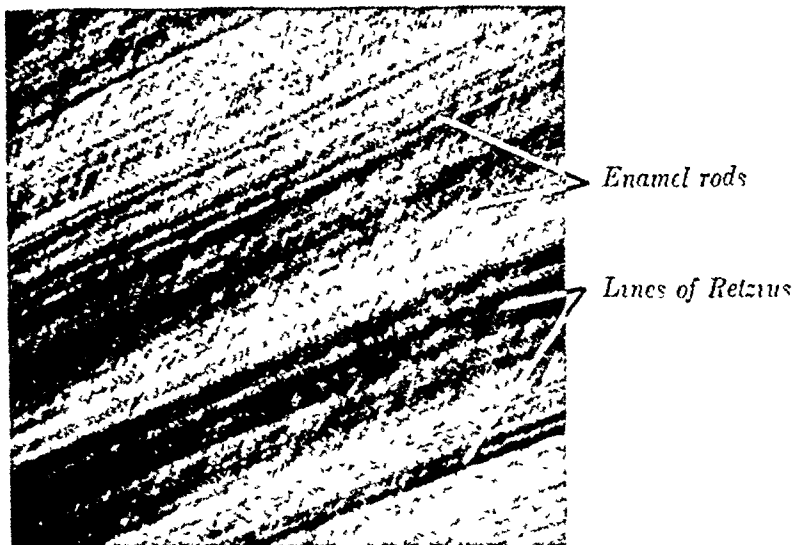


FIG 100 —Ground section of enamel. Position indicated in figure 99. Dark, parallel lines of Retzius of variable thickness cross the smaller and uniformly sized enamel rods at an acute angle. (Modified from Schour, in Cowdry's Special Cytology, Paul B. Hoeber, Inc.)

Obviously the enamel must be very effectively supported from behind, both in the tooth itself and in the jaw. This motive explains many features of dental architecture and why dentists must be experts in mechanical engineering. No bone contains as high a percentage of calcium, nor is so exposed, neither is bone, like enamel, acellular. The enamel is obliged to endure chemical changes and oscillations in temperature imposed by hot liquids, by ice cold ones and in winter in northern climates, occasionally, by air many degrees below zero. This it does usually without noticeable chemical disintegration and without cracking, though both may occur. Another requirement is that the enamel must wear well, for regeneration in the adult is *nil*. It is avascular. Recent investigations on the distribution of radiophosphorus (Sognnaes and Volker, 1941) are significant because they show that more of this is taken up by the outer layer of enamel from the oral secretions than by the more deeply situated enamel indicating the great influence of the fluid in the mouth on maintenance.

Since enamel contains only 1 to 2 per cent of organic matter hardly anything is left after decalcification so that it must be studied in ground section. Enamel is made up of rods and intercellular substance both calcified. The rods are the calcified processes of the ameloblasts. They extend vertically all the way from the free border of the enamel to the dentin. Sometimes they cross one another and occasionally they are twisted ( gnarled enamel ) But the calcification is rarely equally complete throughout the depth of the enamel. It is produced in layers

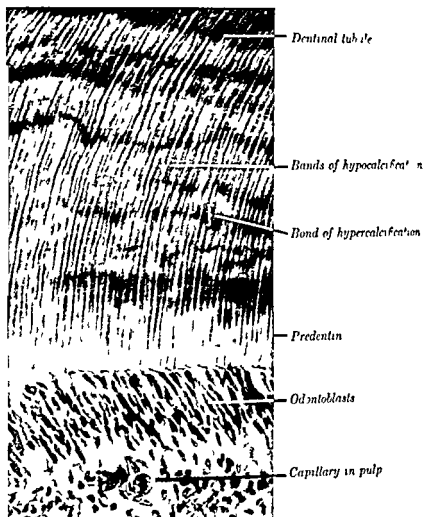


FIG. 101. Dentin and pulp from location marked 101 in figure 99. (Modified from Selzer in Cowdry's Special Cytology, Paul B. Hoeber, Inc.)

the bands of Retzius. These cross the rods distinguished in figure 100 by their finer and more uniform diameter at an acute angle. Each band is marked by deposition of material of brownish color and represents the surface of enamel formed at some particular time in development (Naves 1929). They are accordingly growth lines which stamp nutritional variations on the enamel even more indelibly than at the end of the long bones (Fig. 232).

Dentin is made up of dentinal tubules embedded in a comparatively firm matrix. In ordinary preparations (Fig. 101) the tubules appear to be closely packed together

and to pursue a wavy but parallel course from pulp to enamel. Their uniformity in diameter catches the eye. Viewed in special preparations (Fig 102) each tubule is seen to have a girth near its base of about 4 microns. Proceeding upwards it gives off numerous, extremely fine lateral branches ( $0.3$  to  $0.5\ \mu$ ) at approximately right angles and the main stem may divide several times like a tree trunk. The divisions which result exhibit similar lateral branches and extend almost vertically upwards from the pulp. The length of the tubules is the thickness of the dentin and their number is legion. If those in a single tooth were arranged end to end, they would stretch for miles. Dentin is unexplored territory for those who delight in mathematical calculations. It is alive only by proxy. The odontoblasts of the pulp, which do not reside in it, extend processes, or Tomes fibers, through the dentin in the tubules.

The matrix holds the dentinal tubules in place. It is constructed by calcification of tissue fluid reinforced by collagenic fibers. The latter pass roughly parallel to the dento-enamel junction and, like the osseous fibers of bone, are obscured by mineral matter. The chief mineral constituent of enamel, as well as of dentin, occurs in crystals. It has been identified by roentgen-ray methods as dahlite (Roseberry *et al*, 1931) and as apatite (Thewlis, 1932, 1936). The first designation is more specific for dahlite is a mineral, rich in calcium, belonging to the apatite series. The relatively light bands (Fig 101) are indicative of incomplete calcification. Both light and dark ones are wider, more wavy and less regular than those in enamel (Fig. 100). Next to the pulp there is a relatively uncalcified layer which appears white. This is called predentin.

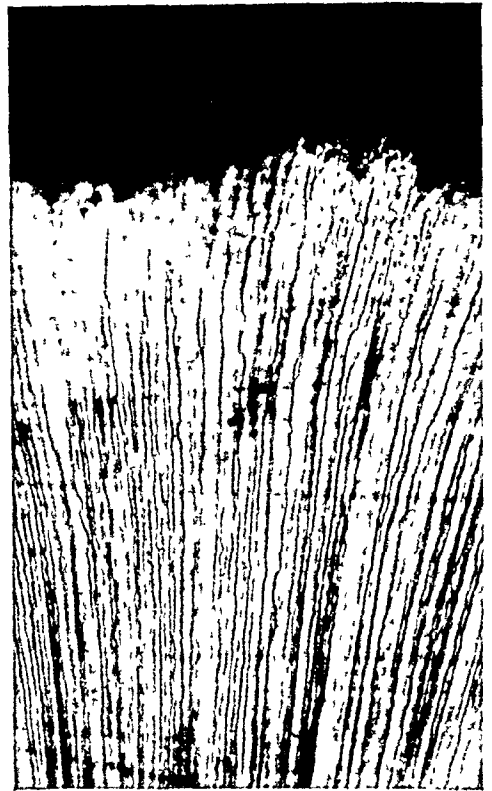


FIG 102 — Ground section of dentin (Specimen kindly lent by Dr. E. P. Brady)

Our knowledge of the functional responses of dentin has been advanced by experiments on animals, particularly rats. It is important to remember, however, that, to compensate for wearing away at the biting or grinding surface, layer upon layer of new tissue is formed in rats but not in humans. Schour and Smith (1934) have clearly illustrated the dark bands of hypercalcification and the light ones of hypocalcification produced daily in the rats' dentin (Fig 103). These are wider and more prominent during administration of sodium fluoride. Schour and Steadman (1935) say that the dark bands are probably laid down by day and the light ones by night. A single light band of hypocalcification, followed by a dark band of hypercalcification, results from a single heavy dose of vitamin D (Schour and Ham, 1934) or parathyroid hormone (Schour, Tweedy and McJunkin, 1931). The effect of the latter is illustrated in figure 104. In human adults provision is made only for maintenance of the dentin in good condition. This depends on the underlying pulp.

The outermost layer of *pulp* consists of the nucleated cell bodies of the odontoblasts whose processes traverse the dentin in the dentinal tubules. These are pressed closely together in a definite stratum at a surface so that they look epithelial though they are in reality of mesenchymatous origin and comparable to the osteocytes of bone. It is this epithelial appearance coupled with difficulty in demonstrating nerve fibers in dentin which is probably responsible for the assertion that some at least of the odontoblasts are sensory cells and that stimuli received by the processes in the dentinal canals are passed on to termini of the fifth nerve and give

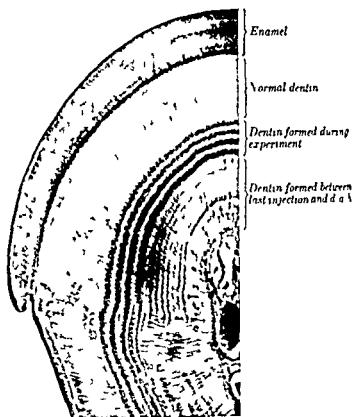


FIG. 103.—Part of transverse ground section of incisor of rat given 4 injections of 2.5 per cent sodium fluoride forty-eight hours apart and killed fourteen days later. Dentin formed during experiment consisted of 4 pairs of light and dark layers each  $32\ \mu$  thick. That formed in the interval between the last injection and death was made up of 14 pairs of light and dark bands each  $16\ \mu$  thick and indicative of normal daily growth.  $\times 60$  (Schott and Smith University of Arizona Technical Bulletin 1934)

the sensation of pain. The odontoblasts are encased in a rich plexus of delicate nerve fibers (Fig. 107) and are backed by loose highly vascularized connective tissue of gelatinous consistency in which elastic fibers are said to be lacking. Details about the blood vessels are supplied by Boling (1942). There are no true eod arteries in multirooted teeth where there is a direct connection between the vessels of each horn. In single rooted teeth hyperemia is supposed to cause closing of the single efferent vein by pressure (self strangulation).

The *cementum* is a thin sheet of calcified connective tissue enclosing the root and interposed between the dentin and the periodontal membrane. As the root

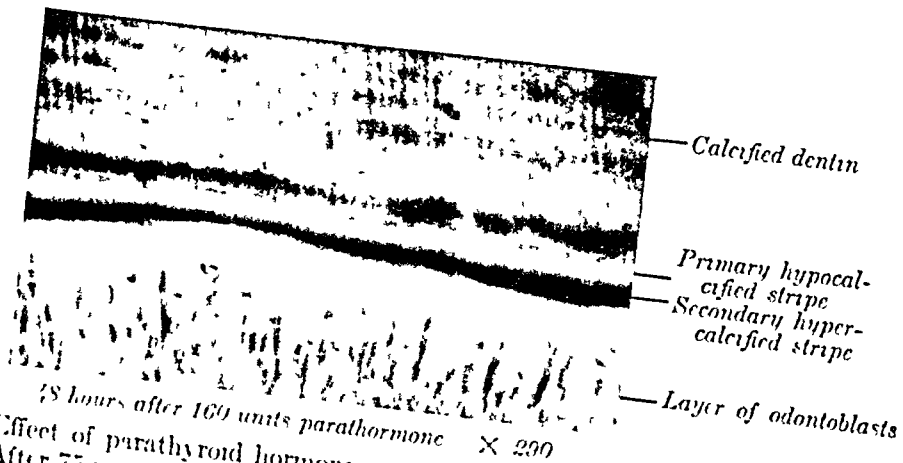
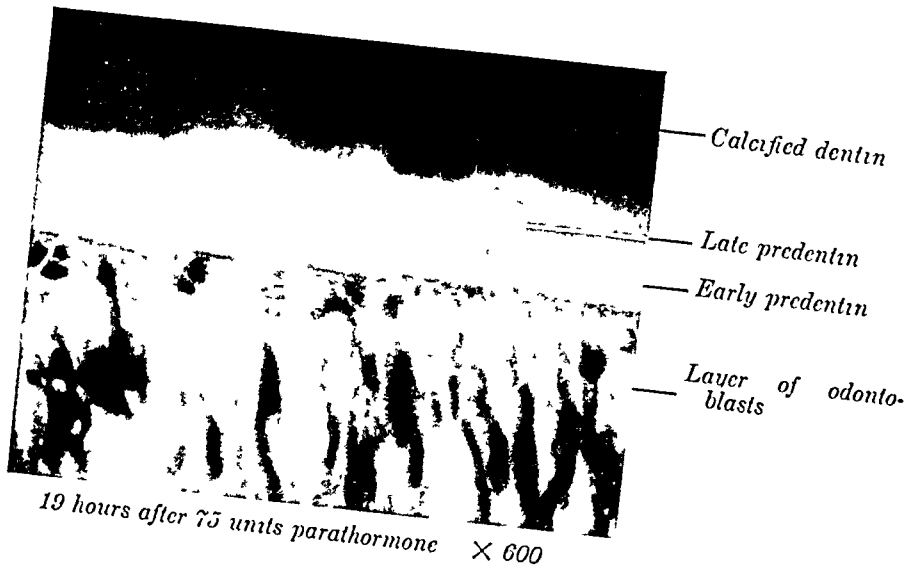
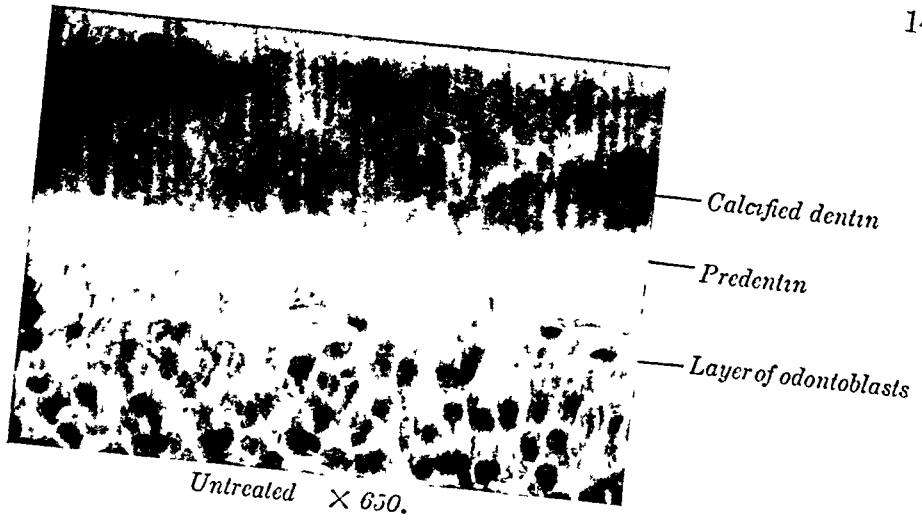


FIG. 101—Effect of parathyroid hormone on the calcification of dentin of the incisor teeth of rats. After 75 units of hormone. Note irregular boundary between early and late predentin. After 160 units and longer period there is first a hypocalcified stripe and then a hypercalcified one  $\times 290$  (From Schour, Tweedy and McJunkin, courtesy of Am. Jour. Path.)

implies it cements the connective tissue fibers of the periodontal membrane to the substance of the root. If the cementum fails, the tooth becomes loose and is soon lost. It is nourished by diffusion from the vessels in the periodontal membrane and new layers which indicate periods of growth (Fig 10a) may be formed from the cementoblasts when required.

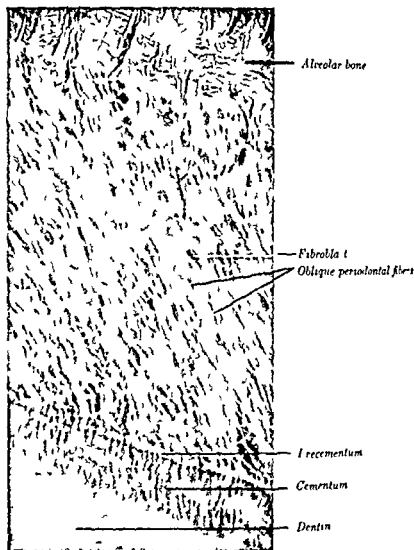


Fig. 10a.—Periodontal membrane about middle of tooth. (Modified from Schour in Cowdry's Special Cytology, Paul B. Hoeber, Inc.)

The *periodontal membrane* (Fig. 10a) is composed of fibrous connective tissue and all the elements which generally accompany it, namely blood vessels, nerves, lymphatics, fibroblasts, histiocytes and so on. The periodontal membrane (1) fills the space between the tooth and the alveolar wall by a cushion of yielding material; (2) by special disposition of its fibers holds the teeth in place yet allows some movement without which they would be destroyed; (3) nourishes the cementum and the highly adaptable alveolar bone and forms both anew when required; (4) gives sensation of touch; and (5) reacts as a very vital tissue to injuries of all kinds.

When the *epithelial attachment* between the gingiva (*i e*, gum) and the tooth is faulty an area of minor resistance exists as in the crypts of the tonsils. Food particles can become lodged in the space between the gum and the tooth and organisms can invade the tissue beneath. It is important to clearly visualize the structures involved. A comparison of young and old gingivæ is illustrated in figure 106.

In the young specimen the enamel has been almost completely removed by decalcification. Only traces of its organic matrix are to be seen. It extended down to the outer limit of the cementum and the bottom of the epithelial attachment was approximately at the cemento-enamel junction.

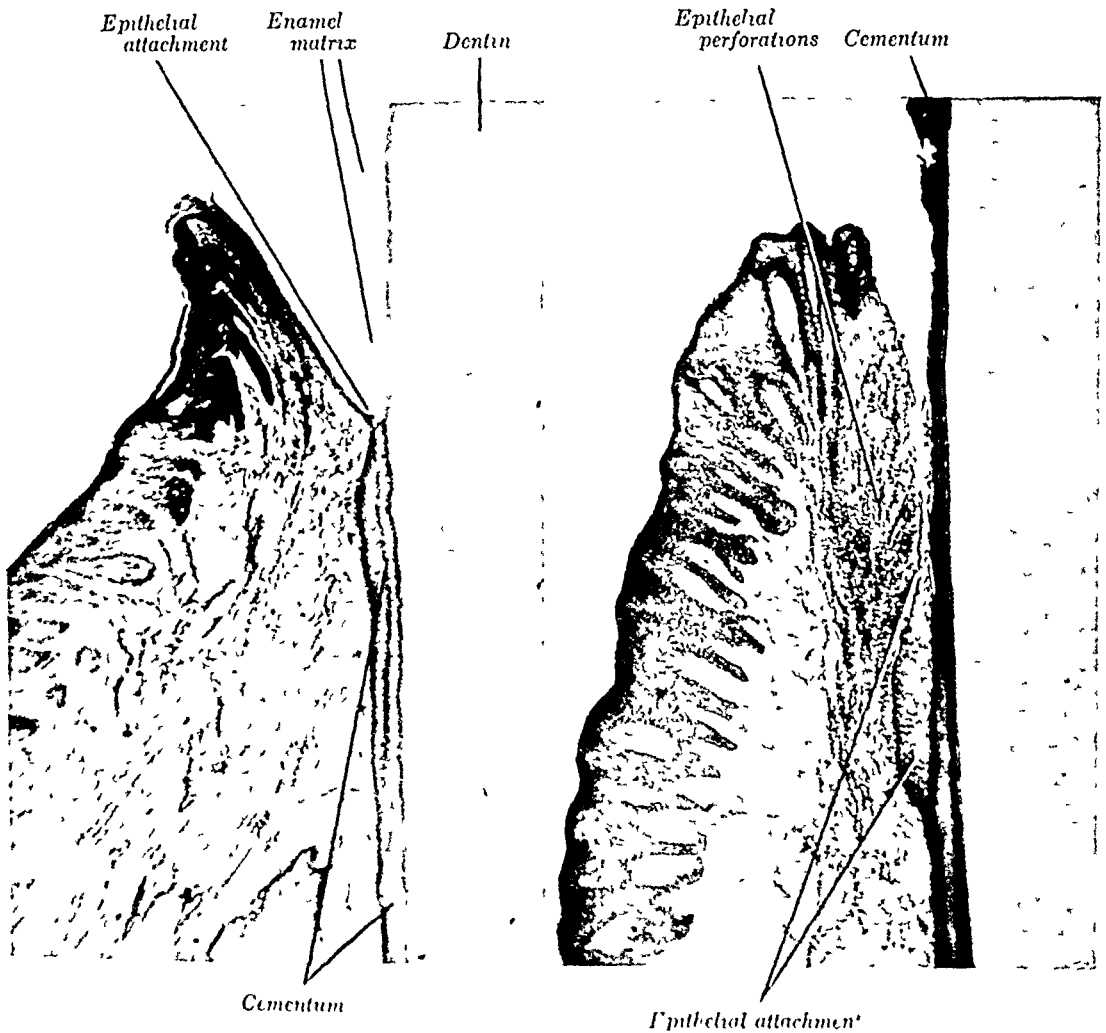


FIG 106 — Sections of young and old gingiva. H & E.  $\times 110$ . (The young specimen was obtained from Dr. L. R. Boling and photomicrograph of the old one is from a preparation of the late Dr. Rudolf Kronfeld.)

In the older specimen, the gum has receded so that the cementum has been exposed. The epithelial attachment has burrowed down (proximally) along the surface of the cementum. The epithelial layer is thickened but is perforated by lines of leucocytes (see Robinson, Boling and Lascher, 1942).

The *lymphatics* of the gingival crevice (or trough) apparently drain down through the periodontal membrane and are joined by others from dental pulp and



alveolar bone (MacGregor 1935-36). Having in mind the large cross anastomoses in lymphatic vessels draining the teeth and jaws of monkeys MacGregor suggests that swollen lymph nodes in humans may not always be due to a focus of infection on the same side of the body.

Windle (1927) has shown that the sensory nerve fibers of the 5th nerve are in 2 groups. The first consists of a few unmyelinated and many small and medium-sized myelinated ones but no large myelinated fibers (Fig. 107). In the pulp Sprenkel (1936) states that all of the unmyelinated fibers are distributed to the blood vessels while the myelinated ones lose their sheaths and form a plexus near the odontoblasts. Riegele (1934) and Sprenkel have observed that twigs from this plexus enter the predentin and perhaps the dentin also. Sprenkel thinks that they extend along the dentinal tubules on the surface, or more probably embedded in the processes of the odontoblasts and figures two kinds of endings: tiny loops and cork-screw formations, the latter in the minority. Both types of endings are sufficiently naked to be interpreted as the termini of pain fibers (see p. 380), and Windle presents some evidence that these small and medium-sized myelinated fibers arise from small and medium-sized cells of the Gasserian ganglion which likewise is consistent with the theory that they are pain fibers.

The second group is, according to Windle, made up of the remaining unmyelinated fibers, and large myelinated fibers and innervates the periodontal membrane and gum. The unmyelinated fibers may pass to the blood vessels as in the pulp but this is not asserted by either investigator. The large myelinated fibers are of the type which may be expected to serve tactile sensibility in the broad sense. Sprenkel has gained the impression that more of the 'heavily myelinated' fibers of the periodontal membrane enter laterally through foramina in the alveolar bone than from below. He has described three types of ending for these heavily myelinated fibers in the membrane: (1) rings situated very near the alveolar bone on the collagenic fibers extending from it to the cementum; (2) terminal reticular endings in association with connective tissue cells; and (3) reticula with radial fibers that penetrate into the cementum and in some cases through it into the dentinal tubules beneath. Sprenkel refrains from any interpretation of the terminal reticula but suggests that all the other endings, including those in the dentinal tubules, are stimulated by deformation and movement of the tooth and serve as receptors for reflex arcs designed to regulate the act of mastication. This view cannot be altogether excluded but the proprioceptive endings in the muscles of mastication would seem to afford sufficient regulation of this function. Obviously we need information on the mechanism for the perception of pain in the periodontal membrane. It may be served by fibers entering from the gums. For innervation see also Lewinsky and Stewart (1936) and Christensen (1940).

The great achievement that marks the maturity of the science of dentistry is not so much the development of marvelous technical skill in combating local infections and in correcting developmental defects as the recognition of the functional integration of the above-mentioned parts to form a whole and of the interdependence of the mechanism with the rest of the body. In the teeth dead and living histological components cooperate to serve the individual to a degree which even exceeds that operative in the outliving epidermis. The brunt of the task of breaking up the food and of mixing it with the saliva is served by the dead enamel. The enamel is supported by the dentin which is certainly at least partly dead though permeable to substances percolating into it from the living tissue of the

pulp. To the sides of the root the living periodontal membrane is attached by special cement substance. This membrane furnishes a slightly yielding attachment in the alveolar sockets of the jaw bones. Even the bony walls of the sockets adapt themselves to functional demands. As is to be expected, the living parts are closely correlated with vital activities throughout the body. This association is decreased in the dentin and barely if at all effective in the farther removed enamel, which latter the body cannot call on for calcium when discharge from calcium reservoirs is required in accordance with the interesting conception of Ham and Portuondo (1933).

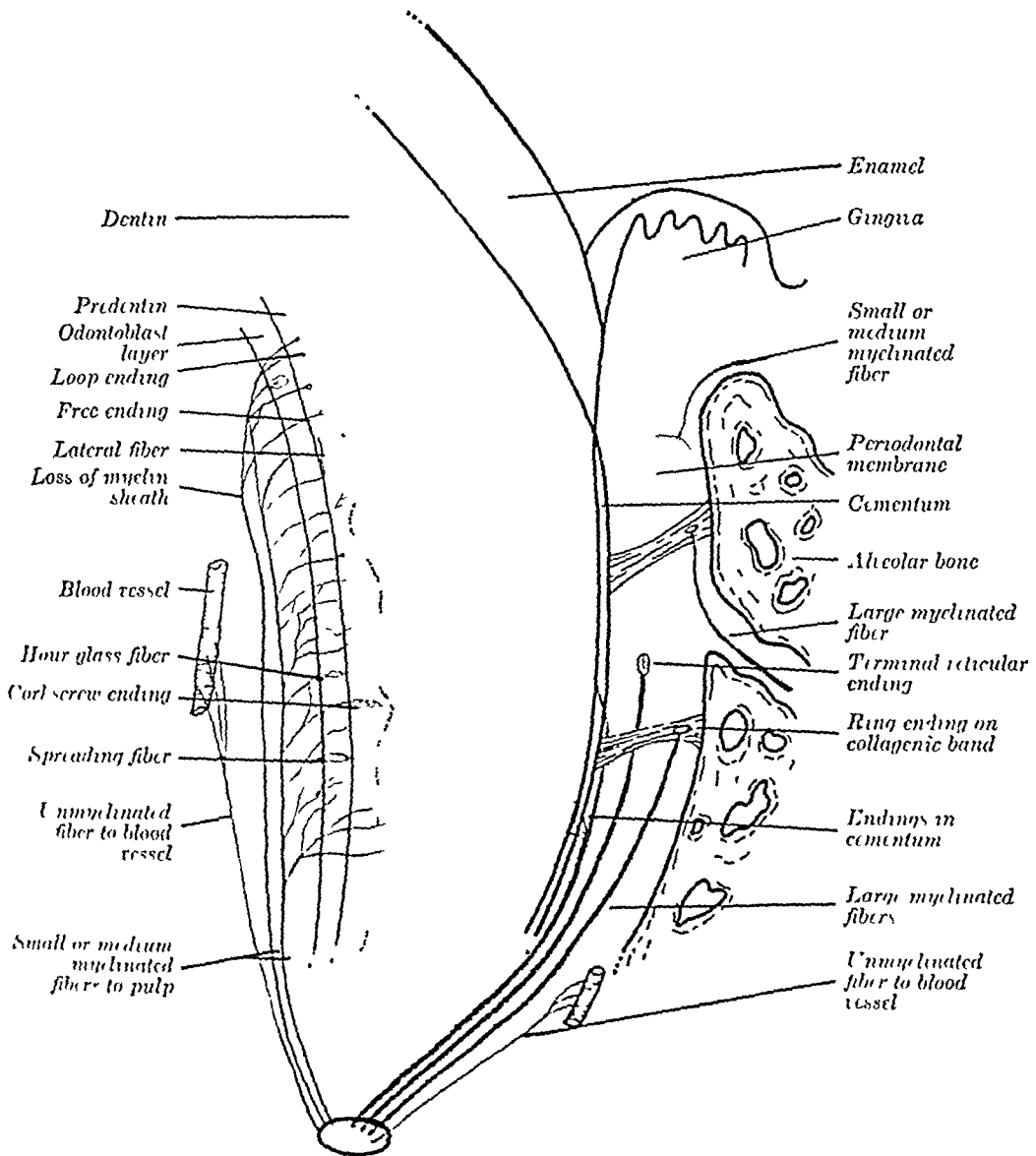


FIG. 107 Diagram of innervation of an incisor tooth.

**Salivary Glands** - There are 3 principal pairs. The sublinguals, submaxillaries and parotids (G. *para* beside, plus *ous* [ōt-], ear). They supply water, ferments including diastase, and mucus to the food which is being ground up by the teeth. They also play an essential rôle in regulating the water supply of the body. As

pointed out by Cannon (1932) when water is needed their secretion made up of more than 98 per cent of water is reduced. Consequently the mucous membrane at the back of the mouth and in that part of the pharynx through which respired air must pass as well as food becomes unpleasantly dry and the sensation of thirst results. The demand for water becomes so urgent that it may lead to a life and death struggle.

It is convenient to describe the structure of the submaxillary and to note in passing some of the chief differences between it and the parotid and sublingual glands. As indicated in figure 108 the secretory epithelium is arranged in alveoli which appear either light or dark in sections stained with hematoxylin and eosin.

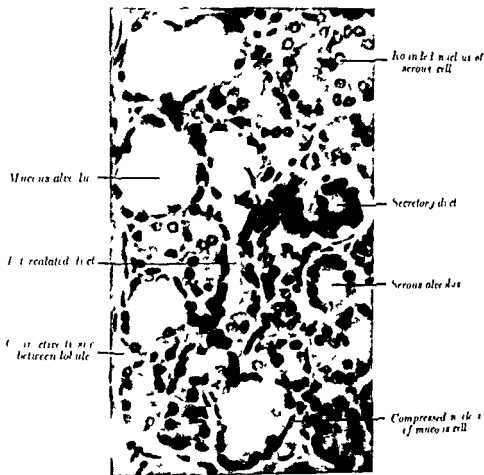


FIG. 108.—Section of submaxillary gland  $\times 400$

The light ones are *mucous alveoli* and they are in the minority. In them the mucogen, which are the secretory antecedents of the mucus, are not preserved. But by appropriate techniques the antecedents can be stained with the result that the mucous cells appear dark and loaded with granules and the serous ones look light. The nuclei of these mucous cells are flattened against the basement membranes of the alveoli. In the sublingual there are many mucous alveoli and in the parotid few, if any.

The *serous alveoli* are colored more strongly by hematoxylin and eosin and are much more numerous. The nuclei of the secretory cells are roughly spherical and

their cytoplasm is colored with eosin. When special methods are employed secretion antecedents, in the form of "zymogen granules" are revealed in them. Sometimes the serous cells do not form a complete serous alveolus but are applied as a "crescent" or "demilune" to one side of a mucous alveolus. This is indicated in the figure 108 in which the serous cells of the crescents are distinguishable from the mucous cells by their spherical nuclei and deeply staining cytoplasm. In the sublingual gland the crescents are more numerous and in the parotid they are absent. The structure of serous (zymogenic or albuminous) cells is considered in detail in the chapter on the pancreas.

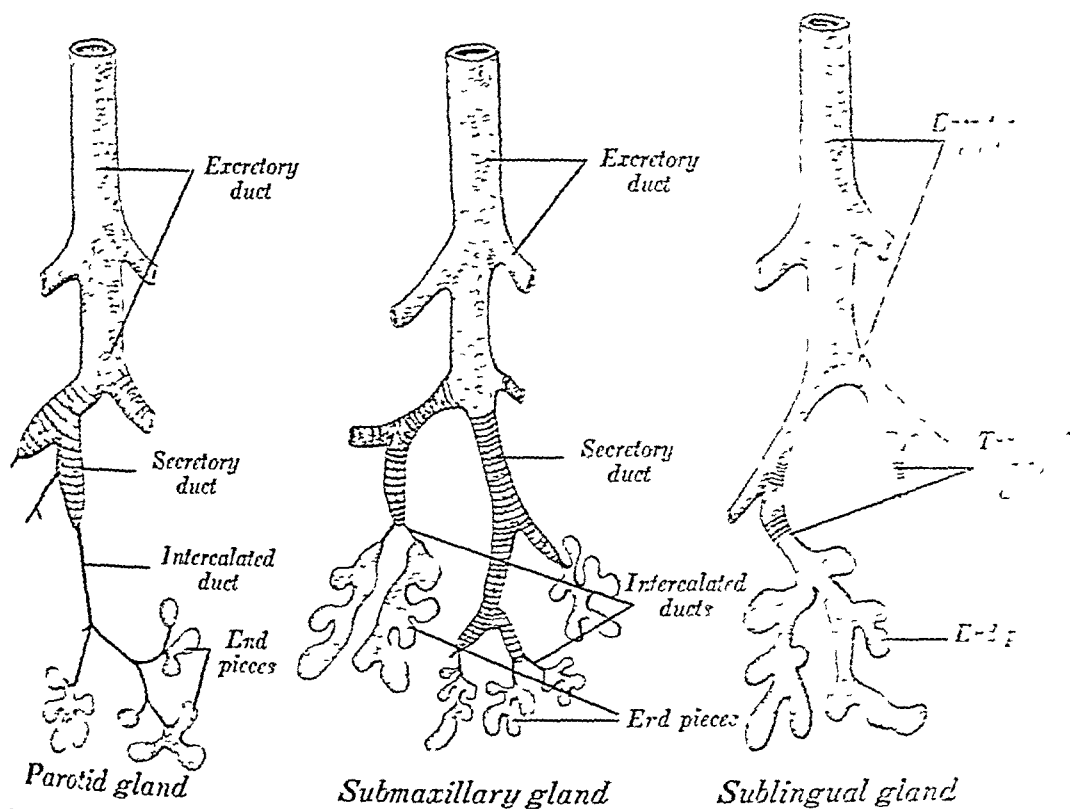


FIG. 109.—Diagram of salivary glands. (Redrawn and modified from Bremer: *Histology* [Lewis and Stohr], P. Blakiston's Son & Co.)

Both types of alveoli discharge into narrow, thin-walled ducts which are called "intercalated" because they are interposed between the alveoli and the larger more conspicuous secretory ducts. These *intercalated ducts* are seen to better advantage in the parotid in which they are much longer. They are absent in the sublingual. In searching for them, a lumen first catches the eye which is clearly that of a delicate tube, not of a rounded alveolus. The cells which border the lumen are short and of about the same height as their nuclear diameter. Their cytoplasm looks clear and stains but faintly.

The *secretory ducts* cannot be easily overlooked. They are much larger and longer. The lining epithelium is one cell thick but the cells are large, their nuclei are rich in chromatin and spherical and their cytoplasm exhibits intensely acidophilic striations, directed toward the lumen. Some writers call these ducts, striated tubules. There is evidence that water, from the surrounding tissue fluid is passed through their walls into the lumen and Maximow and Bloom suggest, calcium salts. Only traces of ducts, marked by this distinctive cytoplasmic striation, are

found in the sublingual but they are abundant in the parotid. Nothing of the kind occurs in the pancreas. As the secretion leaves the secretory ducts and moves toward the mouth it enters larger *excretory ducts* which are not striated. The kinds of ducts of the three glands are represented in figure 109.

The salivary glands enjoy abundant blood and lymphatic supplies which however show no special features. There is no evidence of hormonal regulation. Control is effected by the nervous system. It may not be simply a question of vasoconstriction or vasodilation because, as Stormont has demonstrated in the rabbit's submaxillary, both sympathetic and parasympathetic fibers pass directly to the epithelial components and are distributed differently.

To illustrate the effect of fright on salivary secretion Cannon referred to the experience of Dr. Harvey J. Howard when he thought that he was going to be 'hot by Manchurian bandits'. In describing his sensations Howard wrote: 'My tongue began to swell and my mouth to get dry. This thirst rapidly became worse until my tongue clove to the roof of my mouth, and I could scarcely get my breath. The thirst was choking me. I was in a terrible state of fear.' But he prayed for strength and instantly my thirst began to disappear. In less than a minute it was entirely gone and I was perfectly calm and unafraid.

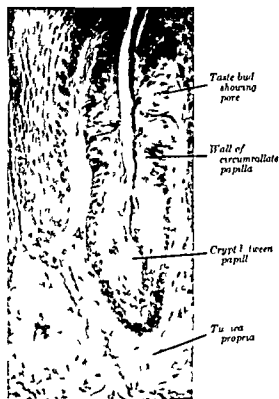
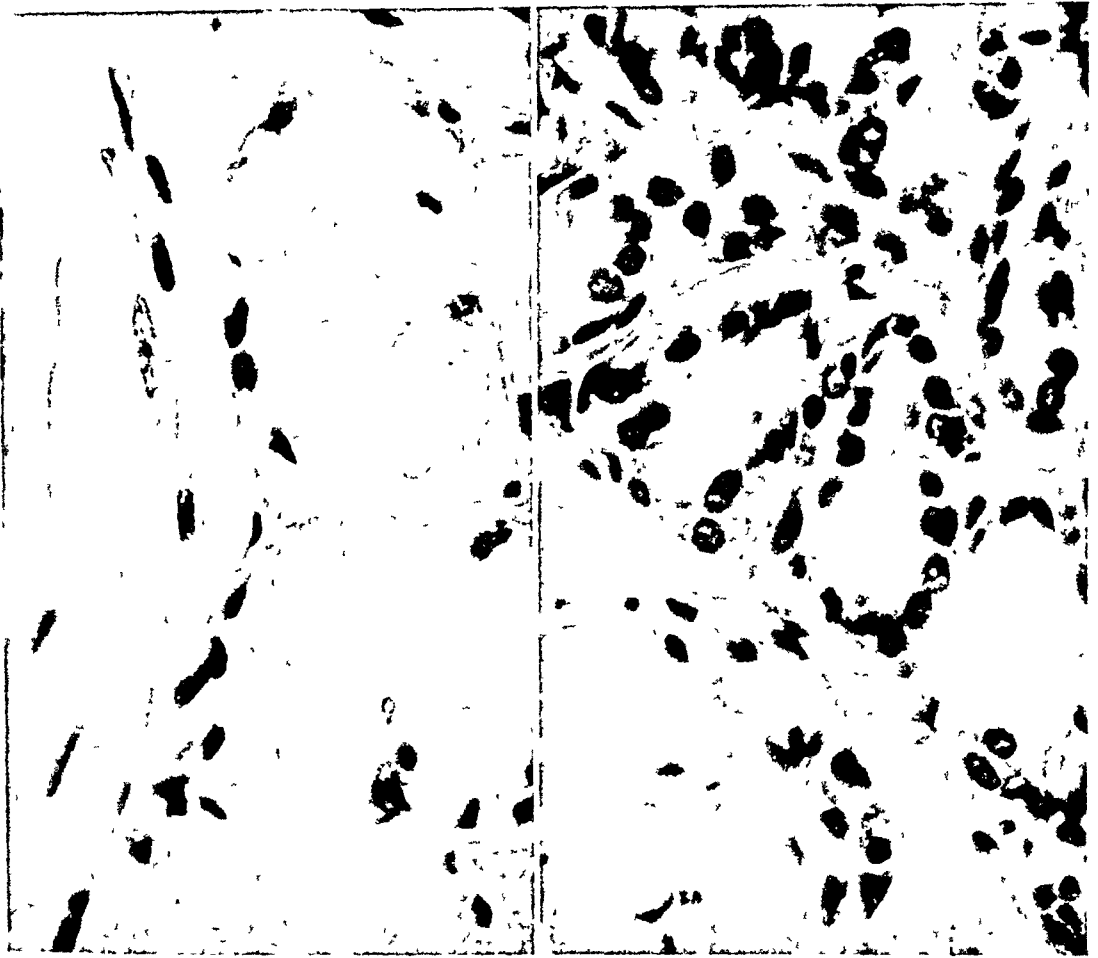


FIG. 110 — Taste buds in tongue

**Tongue** This is the selector of food, the director of mastication and the principal speaker, all in one. The epithelium of the upper surface and sides of the tongue differs from the general epithelium of the oral cavity in four principal ways:

1. It is thicker, constructed of more layers of cells and therefore more protective.
2. It is more tightly bound by connective tissue fibers to the underlying muscle than in any other part of the digestive tract except the gums. In the small intestine

the epithelium may be said to almost float on the fluid and loose tissue separating it from the limiting muscular tunics. This firm association is a useful feature because purposeful movements of the tongue are thereby communicated to the substances being chewed and lodged between the teeth with a directness and force which would be impossible if the epithelium did not follow every change in position of the firm tissue beneath it. In other words, there is no slack to be taken up.



*Mucous alveoli*

*Serous alveoli*

FIG. 111 —Sections of tongue of white female aged thirty-two who died from subdural hemorrhage. Fixation in Kaiserling's Nos. 1 and 3 fluids. H & E. Cells of the *mucous alveoli* (on left) are much distended with secretion antecedent and their nuclei are flattened against the basement membrane, while those of the *serous alveoli* (on right) are not enlarged and possess nuclei which are more or less spherical. Note that nuclei of striated muscle fibers are peripheral when viewed both in longitudinal and transverse section. (Barnes Hospital, No. 10039B, tissue given by Dr. R. E. Stowell.)

3. Its surface is much rougher, due to the projection from it of papillae, which, like the villi of the small intestine, greatly increase the surface area but are not absorptive. The papillae are anchored to the tongue by strong projections up into their cores of connective tissue. There are three kinds: *filiform*, small, pointed and most numerous; *fungiform*, larger, with broad tops, which look redder because their cores are larger and contain more vessels, and *circumvallate*, largest, least

numerous, disposed in V formation at the back of the tongue and each surrounded by a depression into which serous glands empty. Doubtless this irregularity increases the efficiency of the tongue in the propulsion of food.

4 Intra-epithelial taste buds enable the tongue to discriminate between acid, bitter, salty and sweet substances. These structures are located in the walls of the spaces between papillae. Three are partly included in figure 110. They are bluntly pointed barrel-shaped bodies with their pale-staining cells vertical to the epithelial surface. In the upper one of the two on the right the surface is seen to dip into the bud. Close examination may show that the cells are of two kinds: large supportive cells and small thinner looking cells each containing an elongated more deeply staining nucleus and capped with a delicate process extending into the pit. Impulses generated are passed to the brain by the 7th and 9th nerves. Age changes in taste buds have been worked out by Arey, Tremaine and Monzingo (1935). Following injuries these remarkable structures can be regenerated (Arey, 1942).

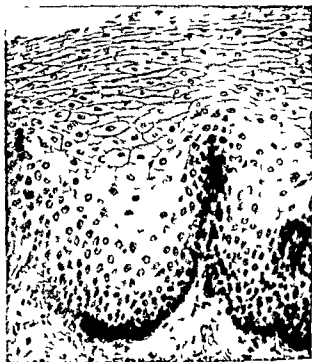


FIG. 112 Lamina propria of an executed negro male aged thirty five years. Formalin Zenker fixation and H & E stain.  $\times 200$

Beneath the epithelium are small mucous and serous glands. In the mucous alveoli represented on the left in figure 111 the cells are engorged with mucinogen which is colored lightly blue with hematoxylin. The cell walls are indistinct and the nuclei are flattened against the cell membranes proximally—that is remote from the lumen and near the surrounding striated muscle fibers. It will be noted that the nuclei of these fibers are near their surfaces.

In the serous alveoli on the right the cells are typically smaller, not swollen with secretion antecedents and possessed of more spherical nuclei. The striated muscle fibers are cut in cross section and the peripheral position of their nuclei is again apparent. Close to the lower margin of the figure a typical plasma cell is visible.

The muscle fibers are disposed in 3 principal series: longitudinal, transverse and vertical. They are innervated by the 12th nerve, branches of which can easily be made out

**Pharynx.**—Structural conditions in the pharynx differ from those in any other part of the alimentary tract because air passes through it regularly (see Respiratory System, p 207).

**Esophagus.**—This tube (*G oiso*, I shall carry + *phagelon*, food) serves, with the aid of gravity, quickly to carry food to the stomach. It is generally examined in sections in the non-distended state and the wall is much folded. The epithelial lining is thick, stratified and avascular (Fig. 112). There is normally little or no absorption through it. The proximal cells, near the blood vessels beneath, are smaller, less watery and stain more strongly than those distally placed. It is these proximal cells that multiply, shift toward the lumen and compensate for loss of cells into the lumen in ordinary wear and tear. A few glands are located in the connective tissue between the epithelium and the muscular tunics. These secrete mainly mucus—the protector and lubricator of the alimentary tract. The muscles of the lower two-thirds are of the smooth involuntary variety.

### SUMMARY

The oral cavity, pharynx and esophagus exhibit a wide variety of specializations because they must perform so many duties. They stand between the environment and the delicate, more deeply lying surfaces of the digestive and respiratory systems. They are the main gateway to the body. The oral cavity provides for the intake of foods, their rejection, if the signal from the taste buds or from the cooperating olfactory terminals of the nose is unsatisfactory, otherwise their mixture with saliva, mechanical breaking-up by the teeth and passage inward by the initiation of deglutition. Failure of salivary secretion causes thirst and calls for more water, which is of all substances the most essential to life. The teeth are constructed in accordance with the best principles of mechanics. The enamel of their cutting and grinding surfaces is the hardest substance produced by living tissues. The tongue and lips utilize expired air in speech. Protection is given by the various secretions and the character of the epithelial lining. The pharynx is adapted for the passage of this partly prepared food and air, while the esophagus serves only as a conductor of the former. Regulation is chiefly nervous, since so many muscles are involved. Maintenance, which is really another form of regulation, is also humoral for adequate supplies of vitamins and hormones and essential materials are required, as by all living cells. The exposed epithelial surfaces, except that of the dental enamel, are replaced when they wear away by generations of new cells produced by cell division in the deeper layers which are backed up by vivifying blood vessels that lie beneath.



## CHAPTER X

### LOWER ALIMENTARY TRACT

Food and drink are passed quickly through the esophagus into these lower segments of the alimentary tract after they have been tested, prepared and found desirable in the oral cavity. Because this is a pleasurable experience adequate time is usually devoted to it. Thereafter digestion and assimilation become unconscious activities which are carried on with less need for protection of the subepithelial tissue fluid. The thick stratified rather impermeable lining epithelium of the upper stretches gives way to a single layer of cells which is continuous to the anus.

**Abdominal Cavity**—Within this cavity the stomach and about 20 feet of intestine as well as other viscera, are accommodated. Because of changes in their volumes easy shifting of surface on surface is essential. This is supplied by a serous membrane the peritoneum (G. *peritoneo* I stretch over).

It is helpful for the students before beginning microscopic study, to make a fluoroscopic examination of the movements of the stomach and intestines in one of their number and to get an idea of the gross relations of the peritoneal cavity from textbooks and cadaver.

Parietal (or wall) peritoneum is applied to the inner abdominal wall while folds of visceral peritoneum (mesenteries and ligaments) extend out from the wall invest the most movable of the viscera and carry to them blood vessels, nerves and lymphatics in the loose connective tissue between their two sheets.

The *omentum* is a particularly large peritoneal fold that reaches downward from the stomach and partly covers the intestines. It has been referred to as the policeman of the belly by those who think that it is protective. According to Pointer (1928-29) the omentum can even "plug a hole in the stomach." Rothenberg and Rosenblatt (1942) have made fluoroscopic observations on dogs in which clips and thread have been placed in the omentum. They find that it exhibits no intrinsic movement in response to the insertion of foreign bodies in the peritoneal cavity and does not take part in the localization of organisms. Neither does it regenerate in dogs (Webb and Simer 1940). Webb and Simer (1942) have contributed valuable data on omental lymphatics.

An excellent and well illustrated account of the structure of intestinal peritoneum has been contributed by a Russian investigator (Baron 1941). The peritoneal surface consists of a single layer of smooth mesothelial cells. Since the cement substance between them can be easily blackened by aqueous solutions of silver nitrate it is a simple matter to distinguish their outlines (Fig. 113).

Immediately beneath the mesothelium is a homogenous looking basement membrane. This Baron has been able to isolate. It is exceptionally thick (8 to 10  $\mu$ ) in the serous coat of the stomach and is supported everywhere by connective tissue in which he describes three strata: a superficial wavy collagenic layer, a superficial unoriented elastic network and a deep latticed collagenic layer.

The space between neighboring viscera coated with peritoneum is normally only sufficient to provide room for lubricating films of serous fluid. The source of this fluid is the blood. In coming from the blood stream it first diffuses out through vascular endothelium. When in this extravascular situation it is comparable to many other tissue fluid that have been mentioned in this book. The second is G

is passage through mesothelium and entry into the peritoneal cavity where it is a good example of tissue fluids of the second order, removed by two membranes from the blood stream (Cowdry, 1942). Abnormal increase in peritoneal fluid, to the

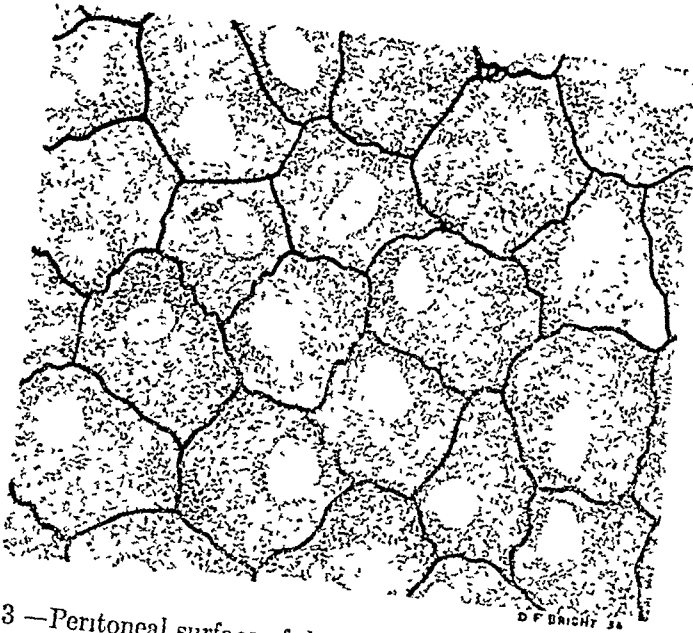


Fig 113 —Peritoneal surface of dog demonstrated by silver nitrate.

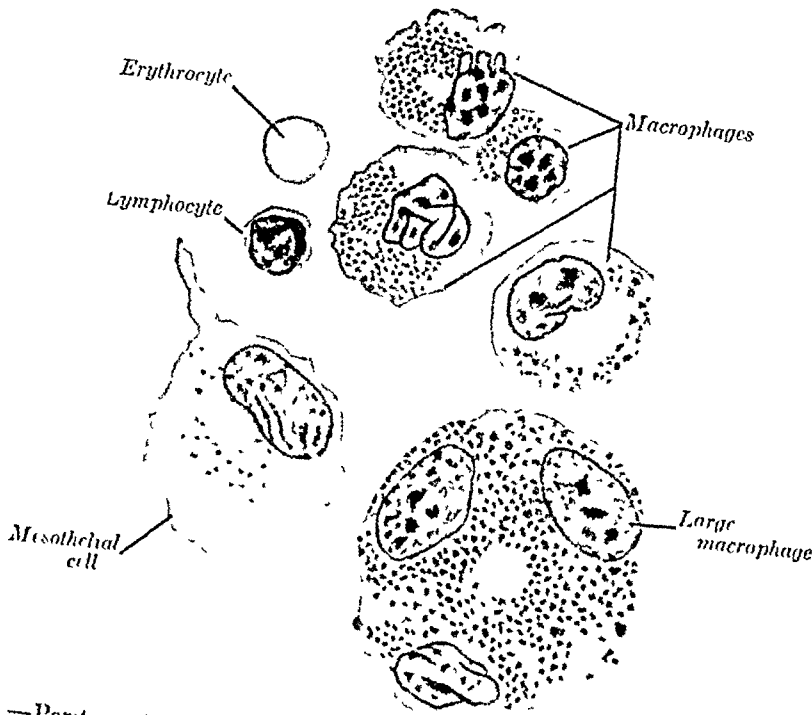


Fig 114 —Peritoneal exudate cells from rabbit injected repeatedly intravenously with lithium carmine (Redrawn from Maximow in Cowdry's Special Cytology, Paul B Hoeber, Inc)

point of its measurement by the quart, is primarily due to increase in permeability of vascular endothelium. The structural reason why it does not remain in this extravascular position is that expansion of this pool of tissue fluid of the first order

is to some extent restrained by the fibrous network. There are then two alternatives: it can be carried off by lymphatic capillaries dilated as suggested by Pullinger and Florey (p. 84) or it can pass on through the highly permeable mesothelial membrane into the peritoneal cavity which is easily distended.

There are some points of resemblance between peritoneal mesothelium and vascular endothelium. The cells of both are closely fitted together and constitute a continuous membrane without demonstrable apertures. Between them is a kind of cement substance. It has been reported that permeability of vascular endothelium is greater along these lines of fusion than through the substance of the cells (p. 67). The same may hold for mesothelium. Baron has emphasized the fact that when mesothelium is stretched by distention of an organ covered by it the cells spread over a larger area, become therefore thinner and the previously wavy lines of cement substance between them are ironed out and stretched. When a capillary dilates the endothelium thins and is known to become more permeable. Probably the intercellular lines are similarly straightened. With stretching of mesothelium a like increase in permeability is to be anticipated.

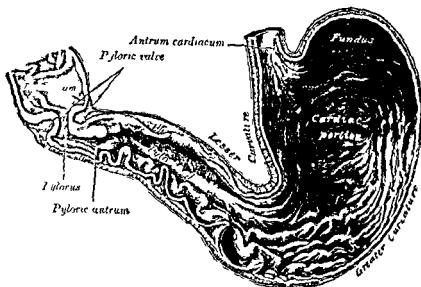


FIG. 113.—Interior of the stomach (Gray's Anatomy)

Regional peculiarities exist in mesothelium as well as in endothelium. Thus the cells covering the ovaries are not flattened but cuboidal even columnar in shape; the reaction of mesothelial cells covering the spleen to vital dyes differ from the rest and absorption from the omentum is particularly rapid. It is in the omentum that fat is most likely to accumulate. Cunningham (1926) found that on irritation the limiting cells become first cuboidal then columnar, and that a considerable number desquamate into the lumen. The defense is by phagocytosis as in the alveoli of the lung. Here also there is controversy as to the origin of the phagocytes. Some identify them with the freed mesothelial cells and others with invading macrocytes. Blood cells of other sorts also enter the area. The responses following intraperitoneal injections of egg albumen and other substances have been investigated by White (1951) and after injury caused by a single erythema dose of roentgen ray by Mitchell (1955) both in the omentum. The phagocytes appear even after intravenous injections (Fig. 114). Replacement of an area denuded of mesothelium

is effected by the same hypertrophy and proliferation leading to a spread of new cells over the exposed part. Unless this happens promptly, there is exudation of blood plasma, the submesothelial fibroblasts become activated, connective tissue fibers are formed and adhesions develop. Prophylaxis against adhesions is a very live problem (Boys, 1942). It is important to remember that the peritoneal cavity differs from other serous cavities (pleural, pericardial and scrotal) by potential openings to the outside *via* the Fallopian tubes.

**Stomach.**—Memory of microscopic anatomy soon fades unless it is related to gross anatomy and both are actively used together in the interpretation of function. The proper approach is to see the stomach in motion by fluoroscopic examination, to study the gross structure of a fresh autopsy specimen, to attempt to dissect it and to examine small pieces microscopically, and then to work out the details in stained sections.

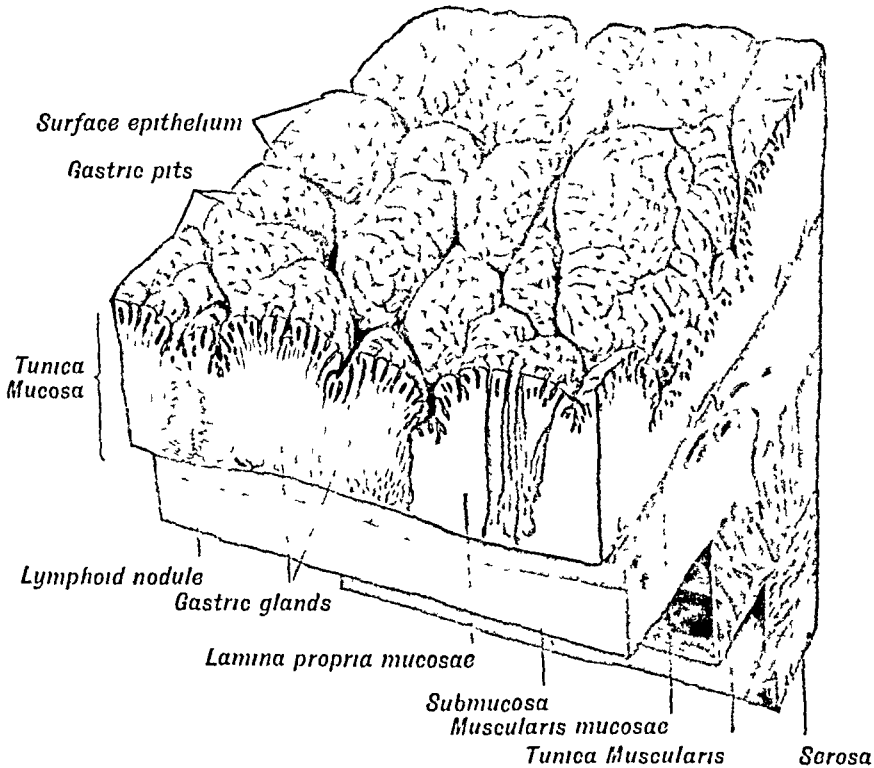


FIG 116 —Microstereoscopic view of stomach wall. Schematic. Crypts, black, glands, gray (From Maximow-Bloom, from Braus, *Anatomie des Menschen*, Julius Springer.)

The internal surface is illustrated in figure 115 and a microstereoscopic view of the wall is presented in figure 116. Passing from within outward note folds in the surface epithelium and the gastric pits in the *tunica mucosa*. Into these pits tubular glands open. Between the glands is the *lamina propria* made up of reticular connective tissue, blood vessels, tissue fluid, etc. A thin layer of smooth muscle, known as the *muscularis mucosae*, separates the mucosa from the *Submucosa*. In the submucosa are the large blood vessels and the *plexus submucosus* (Fig 135) embedded in loose connective tissue and cushioned by a little fat. The *tunica muscularis* typically consists of inner oblique, middle circular and outer longitudinal muscle fibers. The *plexus myentericus* is placed between the two last named. A smooth and slippery *tunica serosa*, composed of mesothelium limits the external surface.

By contractions of the muscles food and drink are mixed up with gastric juice. The mucus-secreting cells which constitute the epithelium of the surface and the walls of the pits are apparently similar throughout the stomach. The mucus is protective. Miller and Dunbar (1932-33) have called attention to relief of patients with gastric ulcers by feeding mucin. It is the cells of the pits which by multiplication and shifting bring about the repair of injuries. A careful consideration of the glands is necessary to localize the sites of production of components of gastric juice. Unfortunately the nomenclature is confusing. There are three kinds of glands, cardiac, gastric and pyloric.

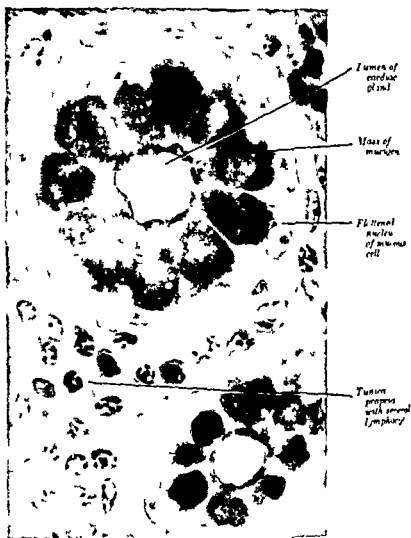


FIG. 117. Cross section of cardiac glands of a monkey. Formalin-Zenker fixation and mucicarmine and iron hematoxylin stain.  $\times 1160$ .

1. The cardiac glands are not, as one might expect, located in the cardiac portion of the stomach (Fig. 115). Instead they are restricted to a small area of gastric mucous membrane extending not more than about 4 cm. from the junction of the esophagus with the stomach. In preparations stained with mucicarmine it can be seen that their cells contain mucigen and that their nuclei are flattened against the

basement membrane (Fig 117) Not infrequently the cells of the deepest parts of the glands contain less mucigen than those nearer the surface

2 *Gastric glands*, or better the *principal glands* of the stomach, occupy about two-thirds of the mucous membrane, that is all the remaining area except for that taken up by the pyloric glands of the pyloric region. They do not, like the cardiac

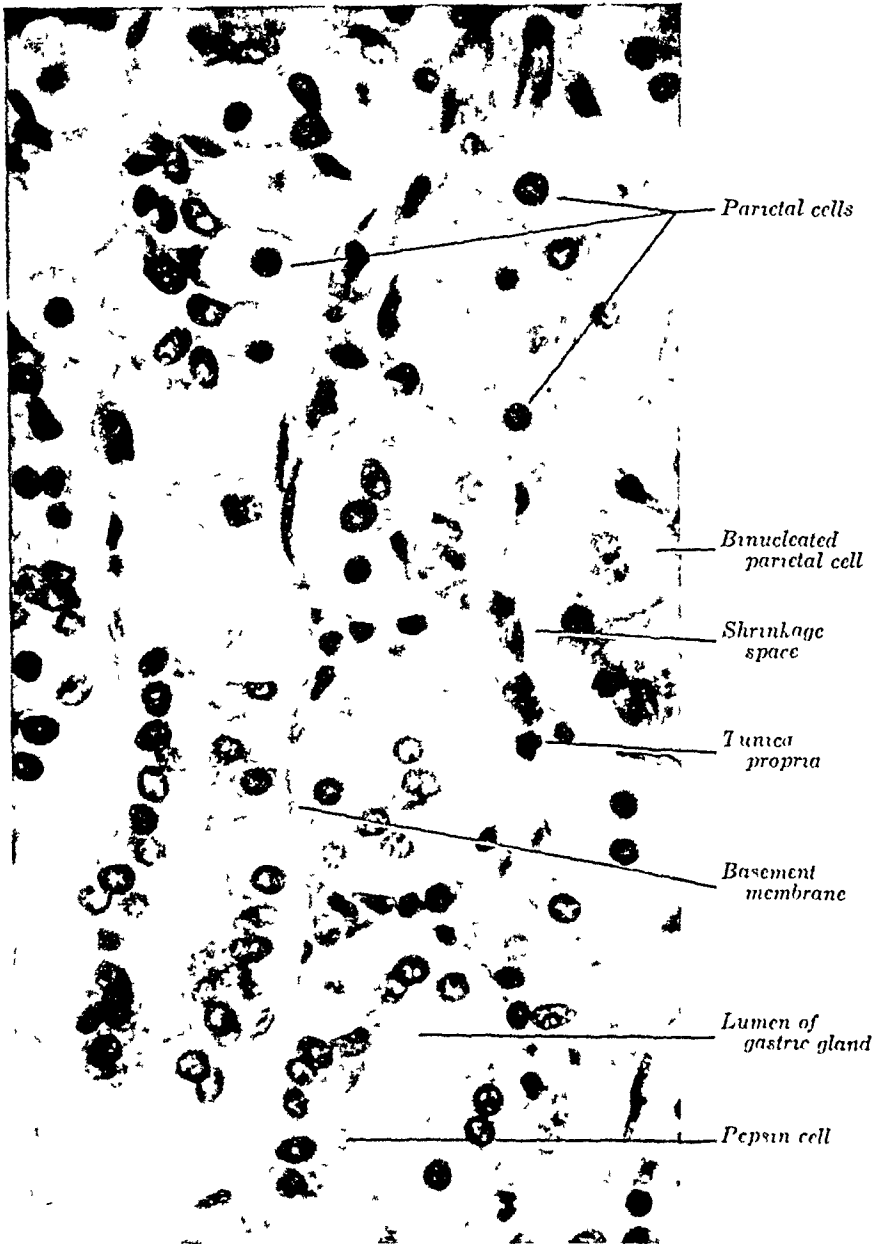


FIG. 118 — Gastric glands of fundus, human Formalin-Zenker fixation, H & E  $\times 580$

glands resemble the esophageal glands, or like the pyloric glands resemble the Brunner's glands of the duodenum They are more distinctively stomach glands than either of the other two because they produce the characteristic secretions of the stomach, pepsin and hydrochloric acid. To call them *fundic glands*, as is commonly done, is misleading since they occupy a much wider area than the fundus

The form of the gastric glands is indicated to the right in figure 116. Their

necks open into gastric pits which are rather short. Such pits are also shown on the extreme right in figure 120. The bodies of the glands stretch a considerable distance parallel to each other vertically into the lamina propria. Their proximal parts near the muscularis mucosae branch and lose the parallel arrangement.

The presence of *parietal cells* in large numbers is the best diagnostic feature of gastric glands. These cells are easily recognizable in ordinary hematoxylin and eosin preparations (fig. 118). Their cytoplasm is acidophilic and stains strongly with eosin. Parietal cells have a rounded, bloated appearance in contrast with their smaller neighbors. As the name suggests they are typically wall cells (*La. parietes* wall) with largest surface next the basement membrane and a wedge-like part extending to the lumen. This relation is further presented in figure 119. But the

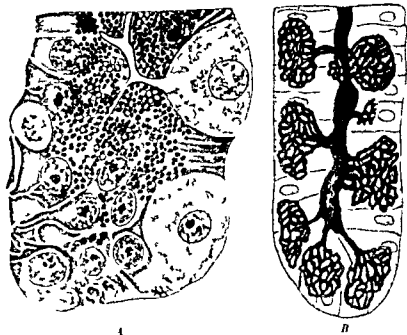


FIG. 119.—Negative (A) and positive (B) pictures of the intracellular canaliculi of parietal cells. A. Section of gastric gland of a monkey stained with iron hematoxylin. The chief cell contains black zymogen granules and the parietal cells exhibit a system of uncolored spaces which communicate with the lumen. (Redrawn from Maxmow-Bloom, *Textbook of Histology*, W. B. Saunders Company.)

B. Positive Golgi impregnation of the canaliculi of the parietal cells of a rabbit. (Redrawn from Henk, 1932, after Golgi, 1893.) (Plenk, *Handb. d. mikr. Anat. d. Mensch.*, v. Mollendorf.)

best way to show parietal cells is by supravital staining of still living mucous membrane with neutral red or raphthol blue, as described by Harvey and Benley (1912). By special techniques canaliculi can be demonstrated within their cytoplasm which converge and empty into the lumen. These are the cells responsible for the production of hydrochloric acid. Curiously enough in monkeys (Cowdry and Scott, 1936), some humans (Doenges, 1938) and several other species they are also the homes of apparently benign spirochetes.

Another characteristic of gastric glands is the presence of many body cells which certainly manufacture pepsin and had better simply be designated *peptic cells*. Figure 120 is instructive. It shows in the first photo a section of gastric glands in which the secretion antecedents have been specifically stained.

They are so abundant that the cells containing them appear black. By contrast the parietal cells, also located in the bodies of the glands look white. Repeated histamine injections cause an active outpouring of gastric juice of high acidity but poor in pepsin. Microscopic examination shows (second photo) that there has been no depletion in the amount of intracellular pepsinogen (Bowie and Vineberg, 1935). But vagal stimulation brings about such a marked discharge of pepsin that the cells show little if any pepsinogen remaining (third photo).

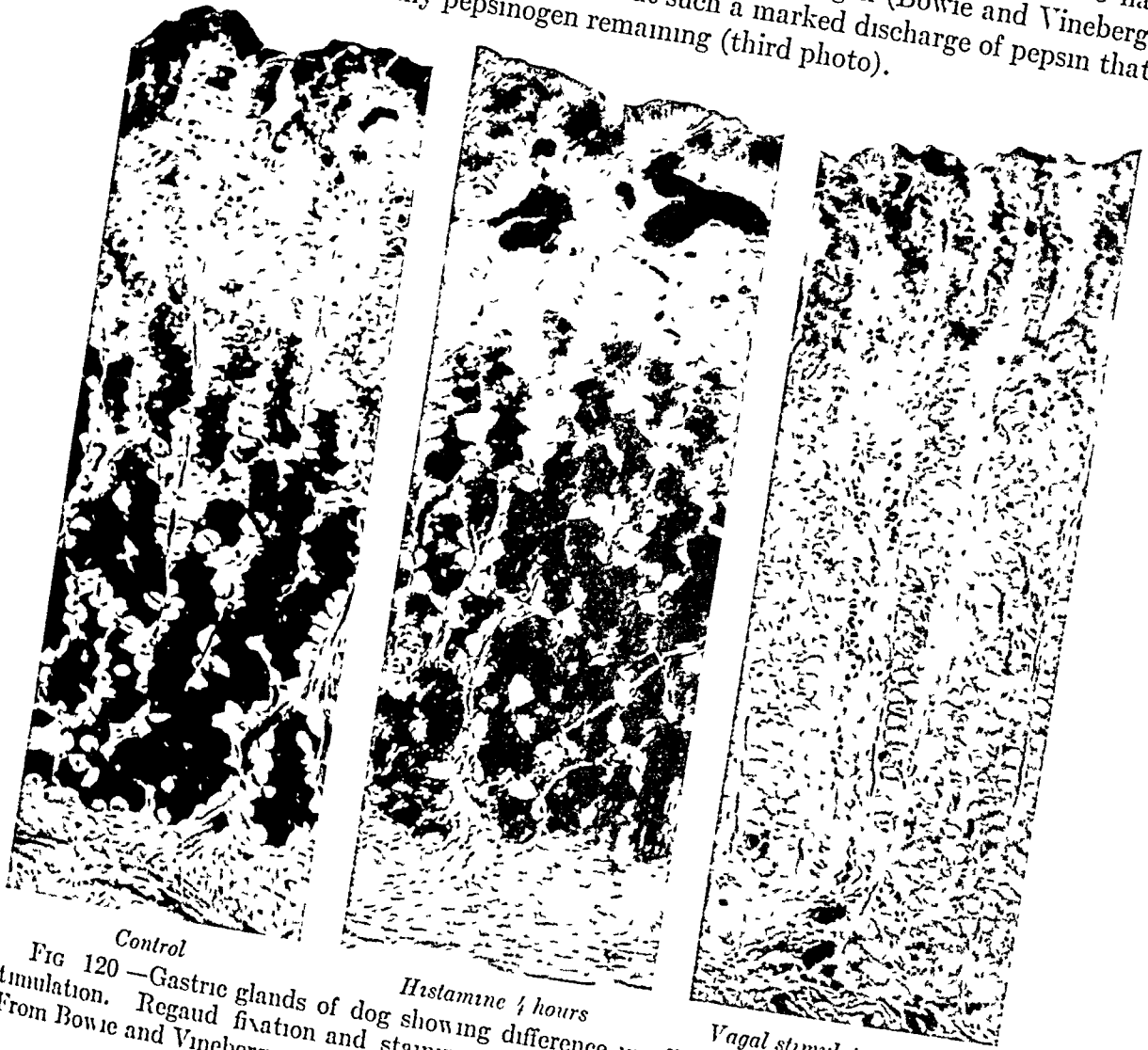


FIG 120 — Gastric glands of dog showing difference in effects of histamine and vagal stimulation. Regaud fixation and staining with crystal violet and orange G  $\times 140$  (From Bowie and Vineberg, courtesy of Quart Jour Exp Physiol)

Linderström-Lang and his associates have devised micro-methods for the estimation of pepsin and other enzymes (see valuable review by Blaschko and Jacobson, Bourne, 1942). Frozen sections  $25\mu$  thick are cut of gastric mucosa, parallel to its internal surface, and their enzymatic powers are compared with their cellular content. It is possible to prove that peptic cells have on the average 17 to 21 units of pepsin. They contain, in addition appreciable amounts of depeptidase and are thought to produce rennin, a milk curdling enzyme quite distinct from "renin" of the kidney.

"Neck chief cells" of the gastric glands are present in relatively small numbers. They contain mucigen (Bensley, 1932), and are to be listed in the large group of



mucous cells. A systematic investigation of mucous glands throughout the body has been made by Clara (1940).

Leucase has been measured in the pig's stomach. It occurs in the surface and neck cells of the fundus and pylorus attaining a maximum in the former.

*Pyloric glands* differ in structure from the gastric ones. They are much shorter occupying only about half the depth of the mucosa, the other half accommodating gastric pits which are longer than in any other region of the stomach. The secretory cells produce mucus and resemble at least superficially those of the gastric glands and of Brunner's glands of the duodenum. In pigs these cells exhibit more than four times as much peptidase activity as the peptic cells of the gastric glands and approximately 1/10 as much pepsin. But typical peptic cells are like parietal cells generally absent in pyloric glands.

Though these three types of glands have been described individually, gradations between them exist. In the zone of transition from cardiac to gastric glands the pits become shorter, the bodies of the glands become straighter and peptic and parietal cells increase in number, while in that from gastric glands to pyloric ones the pits become much deeper, the glands shorter and less straight and the peptic and parietal cells disappear.

Mention has been made of the cells concerned in the production of mucus, hydrochloric acid, rennin, pepsin and dipeptidase. *Lipase* occurs in the surface epithelial cells and in the cells lining the gastric pits in about the same concentration in all parts of the stomach. The intrinsic antipermeicous anemia factor and enterochromaffin cells are mentioned later (p. 166).

**Small Intestine**—1 Examine first a comparatively fresh human intestine. Divide a segment by pushing a test tube of appropriate size into the lumen. As reported by Barron the mesothelium can be loosened by simply washing the surface for ten to fifteen minutes in running water. Try to remove it as a sheet and examine microscopically. Strip off the rest of the serosa, then the muscularis noting the direction of fibers and leaving the mucosa intact. Take small pieces of mucosa, mount them in physiological saline solution in the usual manner at low magnification. Finally with dissecting needles pick out individual villi and stain with oil immersion objective. To obtain a clearer concept of single muscle fibers cut moderate intestine on the tube in 15 per cent aqueous nitric acid two to three days. Then separate them (Technique p. 178).

2 Barron's movements of the villi are quickly suppressed by failure of the circulation. Make a wide opening in the jejunum of an anesthetized animal and look with hand lens for the movements described by King and Arnold (1922). King, Arnold and Church (1927) and Von Kries (1935-39). It should be possible to see rhythmic shortening lengthening and lateral waving. The length and shape of villi can be altered in rats by unbalanced diet (Wier 1942).

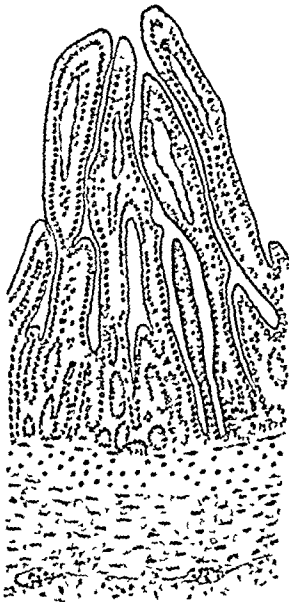
3 Demonstration of the absorption of fat has been mentioned (p. 84).

The appearance of the small intestine depends upon whether it is in the contracted or distended state (Fig. 121). Moreover most microscopic preparations of human intestines are faulty because during the gross examination they have been washed out with water and subjected to considerable trauma. The tips of the villi are sometimes even scraped off.

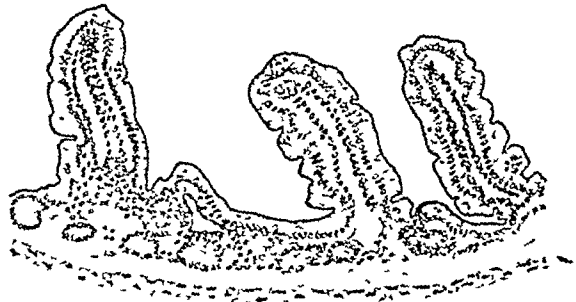
The general structure easily visible with a hand lens is illustrated in figure 121 which should be compared with a similar figure of the stomach (Fig. 116). The internal surface is less smooth because it has many delicate processes extending into the lumen (villi tufts of hair). Intestinal glands (pits, crypts or glands of Lieberkühn) open at the roots of the villi. The reticular framework both within the villi and between the glands is again known as the lamina propria. Circular

folds of mucosa (*plicæ circulares*) project into the lumen. Thin sheets of *muscularis mucosæ* extend up into the *plicæ* and beneath them is the *submucosa*. Then come the circular and longitudinal muscles, which are thinner than in the stomach and slightly spiral in arrangement (Carey, 1921a, Goerttler, 1932). No layer of oblique muscle is present. A serosa limits the wall.

The small intestine, like the stomach, is divisible into three segments the minute structure of which grades gradually one into the other. Throughout its extent the small intestine differs sharply from the stomach in the possession of villi and in the char-



Contracted



Distended

FIG 121 —Shows how different the structure of the small intestine is when strongly contracted and normally distended with food material  $\times 80$  (Redrawn from Johnson, courtesy of Am J Anat.)

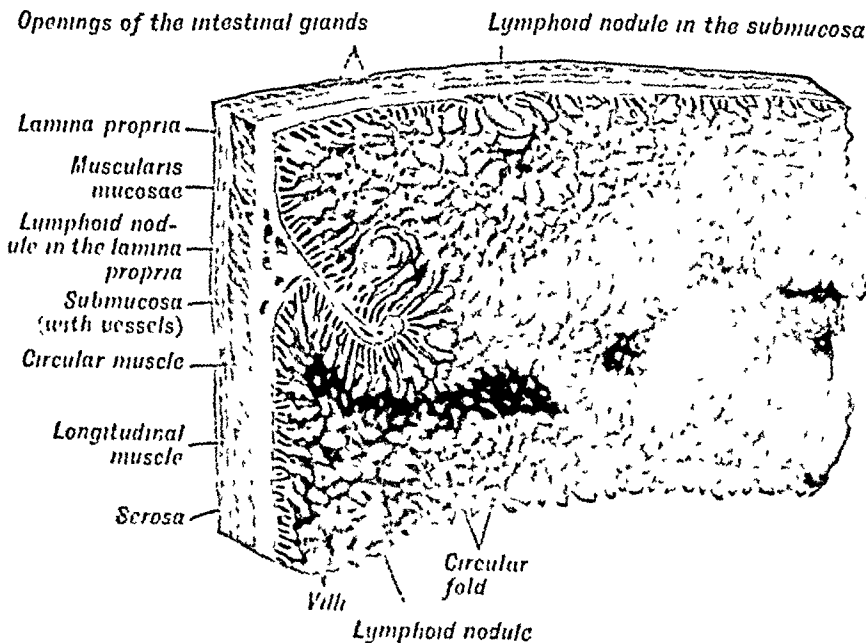


FIG 122 — Stereomicroscopic view of intestinal wall. Schematic. (From Maximow-Bloom, redrawn from Braus, *Anatomie des Menschen*, Julius Springer.)

acter of the *surface epithelium*. In the stomach the surface cells, except for occasional dead and dying ones, all look alike and are engaged in the same process of gradual secretion of mucus. In the intestine, on the other hand, the cells are

divisible into two categories. The first form mucus but before its expulsion *en masse* on completion of the secretory cycle they become greatly distended (Goblet cells). They are illustrated at low magnification in figure 128. The second



FIG 123 — Mitochondrial changes during fat absorption by the intestinal epithelial cells of white mice revealed by the Champy Kull technique. A Normal control contains many mitochondria. B and C Two stages of absorption. Note decrease and granulation of mitochondria, increase in fat (large gray spherules) and movement of nuclei away from the lumen. (Redrawn from Weiner, *Ztschr f mikr anat Forsch*.)

are more slender, are characterized by the possession of an evenly striated (cuticular) border next the lumen and serve primarily in absorption from the lumen of water and various products of digestion (absorptive cells). Water is not absorbed by the gastric mucous membrane as nauseated persons know full well, whereas alcohol is

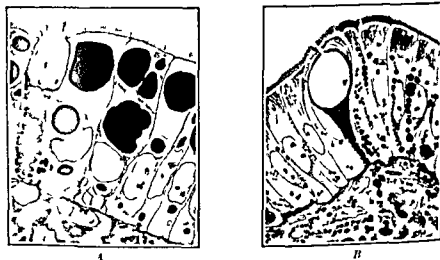


FIG 124 — Influence of vitamin B deficiency on duodenal epithelium of rats. A Received a vitamin free diet. B a diet containing vitamin B. (Redrawn from Mottram, Cramer & Drew, *Brit J Exper Path*.)

always quickly absorbed. Some mitochondrial changes during absorption of fat are shown in figure 123 and modifications in transport of fat in vitamin B deficiency are represented in figure 124. In the absence of vitamins the fat seems to clog the

cells, in the presence of B it apparently passes easily in streams. Such cells are rich in lipase and contain phosphatase. Owing to the fact that they drink up many materials they may also take in enzymes. Methods of micro-incineration and fluorescence microscopy will probably be useful in tracing the absorption of certain substances

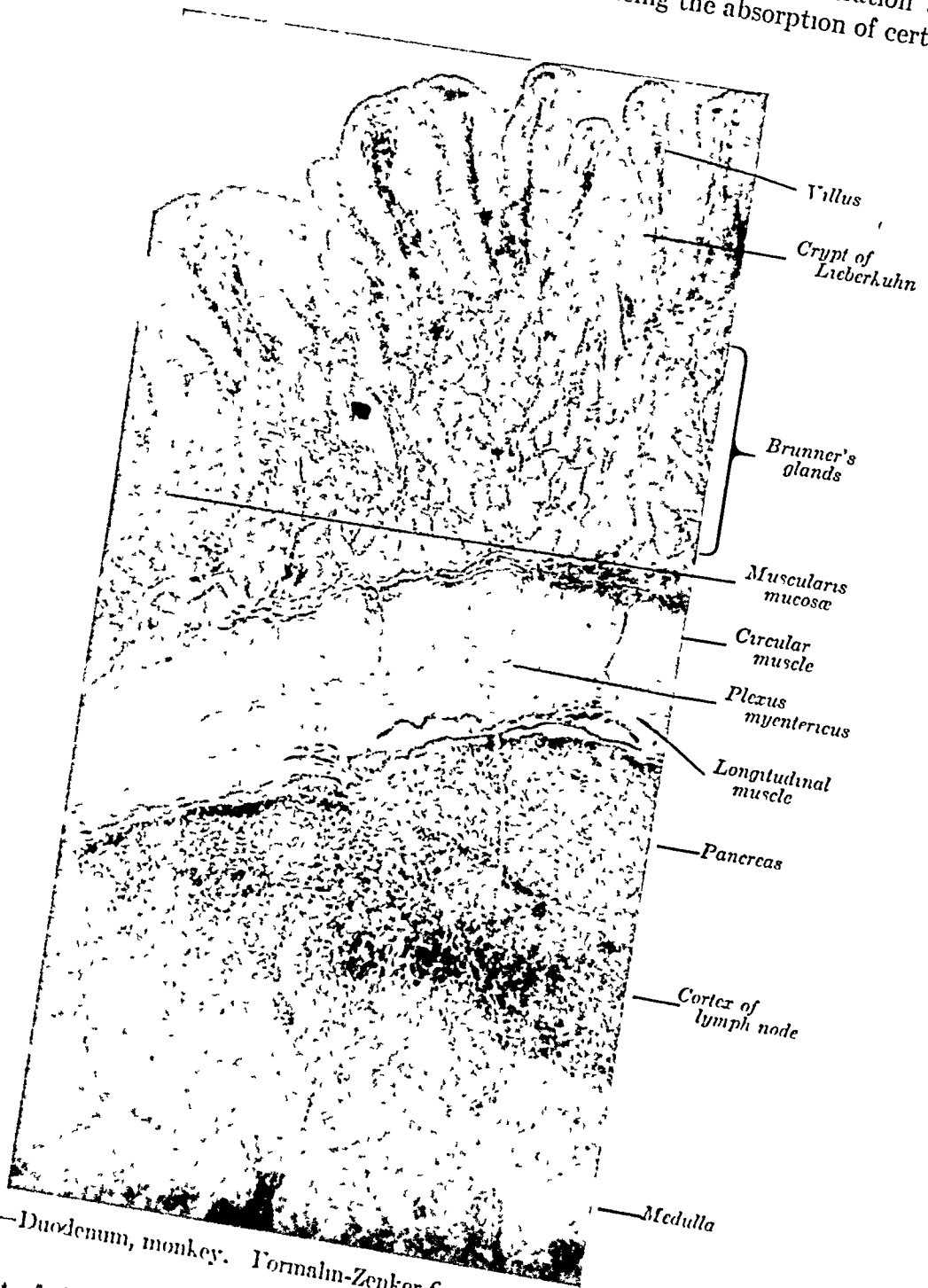


FIG. 125 —Duodenum, monkey. Formalin-Zenker fixation, H & E  $\times 75$

The first part of the small intestine is the *duodenum* so named because some ancient anatomist measured length, like a Scotchman does his whiskey, by finger-breadths (*L. duodeni*, twelve). This is the widest portion of the small intestine and

the only one not connected by a mesentery with the posterior abdominal wall. Part of its anterior and lateral surface is however covered with peritoneum and consequently this part has a serous coat. A feature of the duodenum is the presence of *Brunner's glands* located not like the others in the lamina propria but in the submucosa. Their ducts pass through the mucularis mucosae and discharge into the pits as indicated in figure 125. *Brunner's glands* decrease in size and number as one passes toward the low end of the duodenum. In some sections of the lower duodenum none may occur. The cells of these glands are chiefly *mucin producers* but the *Linderström-Lang* technique reveals the presence in them of some enzymes. They contain a little *pepsin* whereas no *pepsin* is found in the epithelium of the pits and villi and a little more *dipeptidase* and much more *amylase* than the epithelium of the pits and villi. In the rat which has no serous cells *amylase* is not present at the level of the glands (Van Gendren and Engel 1935). (See Robertson (1941) on pathology of *Brunner's glands*.)

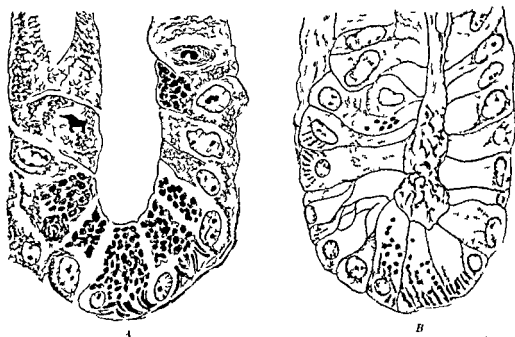


FIG. 126.—Physiological changes in Paneth cells. A Guinea pig after twenty-four hours fast. B same six hours after feeding. Note shrinkage of Paneth cells and decrease in their cytoplasmic granules. (Redrawn and modified from Klein, *Am. J. Anat.*)

At the bases of the pits, especially those into which *Brunner's glands* do not open, are a few *Paneth cells* clearly marked by many large eosinophilic granules congregated in the distal cytoplasm between nucleus and lumen. The granules have the appearance of secretion antecedents (Fig. 126) but the nature of the secretion has not been established. Literature on the subject is critically reviewed by the Macklins (1932).

In addition to these four kinds of cells (goblet and absorptive of surface mucus of *Brunner's glands* and *Paneth* of the pits) is a fifth rather ill-defined variety called *enterochromaffin* because it is intestinal and gives a positive chromaffin reaction (p. 121) not unlike that of the medullary cells of the adrenals. To employ the term *argentaffin cells* is unsatisfactory for the reason that such a wide variety of cells

and cellular components enjoy an affinity for silver. Normally these cells are of extremely rare occurrence but are said to be most numerous in the duodenum near the pylorus. In searching for them in ordinary preparations bear in mind: (1) their parietal position next the basement membrane (Fig. 127); (2) that they occur singly, that their cytoplasm is studded with fine granules, which may have a faint yellow cast in tissues fixed in fluids containing potassium bichromate; (3) that the granules are often concentrated in the cytoplasm distant from the lumen, not as in figure 127, and (4) that figures, useful in their identification, are to be found in papers by Jacobson (1939) and Gillman (1942). These enterochromaffin cells were prominently mentioned in papers by Masson (1928, 1930) in connection with the origin of argentaffin tumors of the alimentary tract. Now, on rather slim evidence, it is claimed that they produce the intrinsic antipernicious anemia factor.

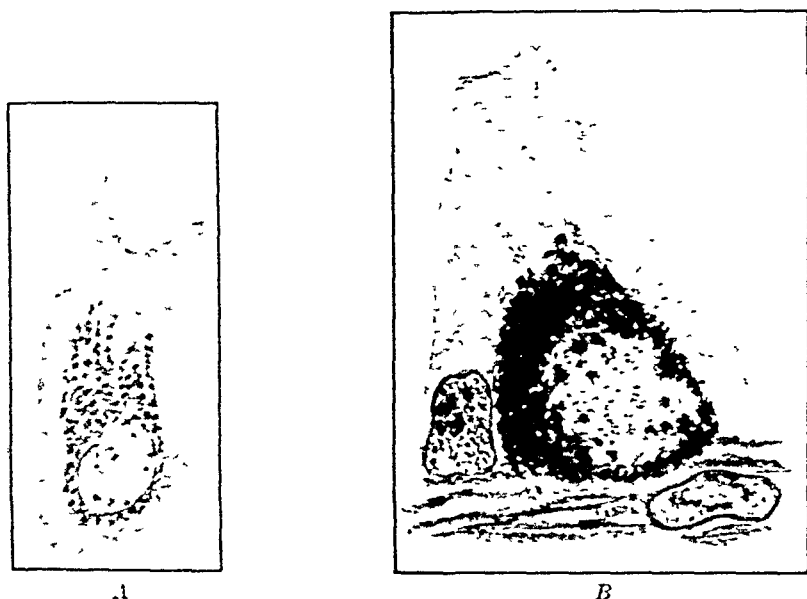


FIG. 127.—Physiological changes in enterochromaffin cells of the guinea-pig's jejunum. *A*, Twenty minutes after injection of pilocarpin, *B*, fifty minutes after similar injection. (Hamperl *Ztschr f mikr.-anat Forsch*)

*Enterokinase*, the enzyme which activates pancreatic enzymes (p. 184), can be traced to the duodenal pits but which cells manufacture it remains a mystery. Glick (1934) has measured *lipase* activity and found it to be greatest in the villi but fairly strong in all parts of the mucosa. That the hormones *secretin* and *cholecystokinin*, are produced in the duodenum is clear but the cells of origin cannot be specified.

The second part of the small intestine is termed the *jejunum* since it is often found empty after death (L. *jejunus*, empty). It is described by gross anatomists as commencing at the duodenojejunal flexure, but its beginning is not sharply marked by a change in microscopic structure of the gut. It includes about two-fifths of the remainder of the small intestine, the lower three-fifths being ileum. Brunner's glands may extend a short distance into the jejunum but they are typically absent. Villi are usually longer and the pits somewhat shorter than in the duodenum.

The third segment, *ileum* (G. *elbeo*, I roll up) is distinctly coiled. It extends to the ileocecal sphincter. Dipeptidase has been measured and is said to be produced

in the Paneth cells. According to Blaschko and Jacobson it should be studied in dogs and cats which they list as not having Paneth cells. Its structure differs only slightly from that of the jejunum. Lymphatic tissue is more prominent and may take the form of Peyer's patches. These are masses of nodules within the tunica propria usually on the side of the intestine opposite to the mesentery and are easily recognizable. In general the ileum is narrower, has thinner wall, shorter less numerous circular folds in the mucosa and is less vascular than the jejunum.

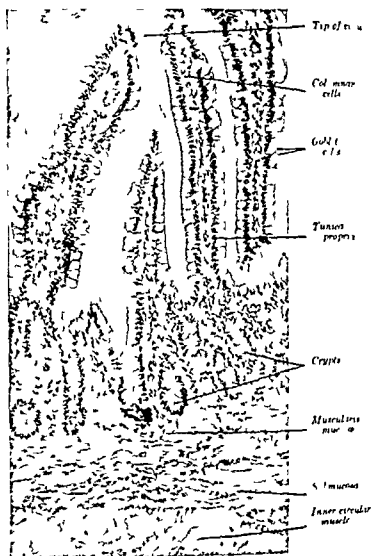


FIG. 128. Ileum of an executed negro male aged thirty-five years. Zenker fixation. H & E stain.  $\times 140$ .

but the pattern of blood vessels is the same (Fig. 129). It is not cricket to ask students on the basis of but a few hours study of the small intestine to identify sections of different parts unless there are present obvious clues as for instance well developed Brunner's glands, Peyer's patches, a clip through the pancreas or the bile papilla marking the opening into it of the common bile and pancreatic ducts or parts of the pyloric or ileocecal sphincters.

Meckel's diverticulum is a finger-like process found attached to the ileum about

3 feet above the ileocecal valve in approximately 2 per cent of individuals. It is a vestige of the original connection through the umbilical cord with the umbilical vesicle, is lined with epithelium and is seldom more than 3 inches in length. As a blind pouch, which may loop about the ileum causing strangulation, become

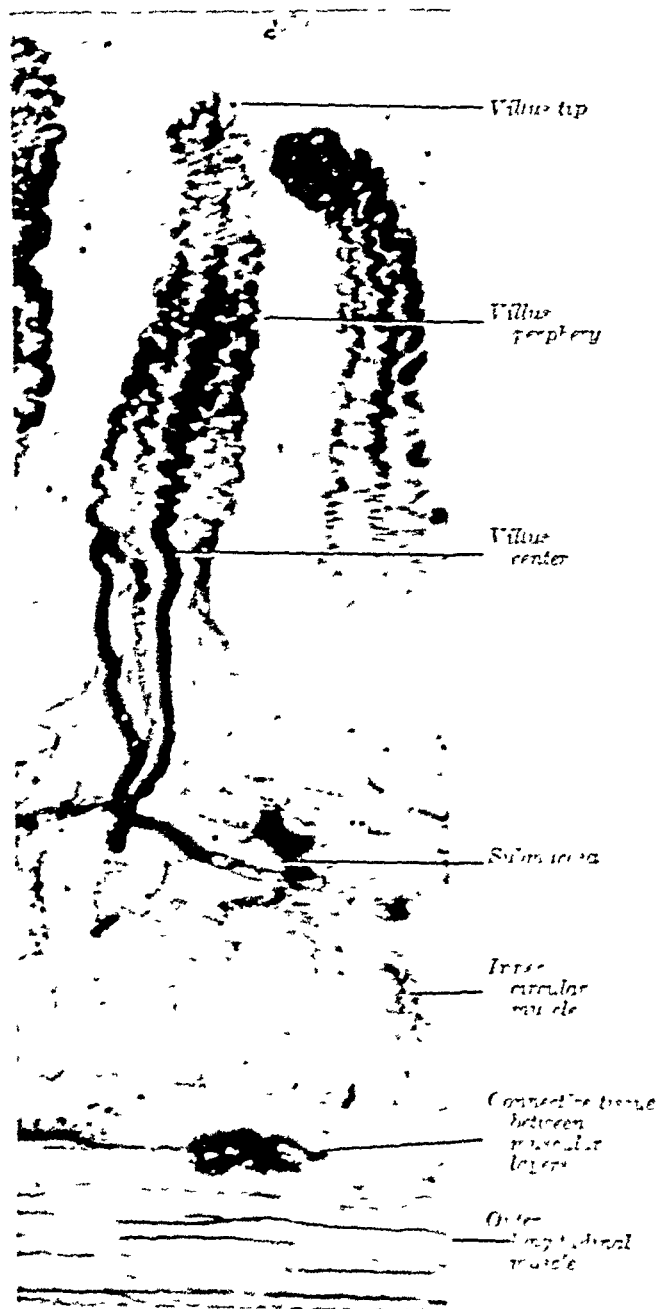


FIG. 129.—Unstained section of wall of human duodenum; blood vessels had been injected with carmalum-gelatin.  $\times 145$ .

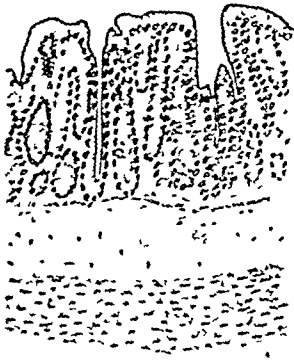
infected or inverted into the intestinal lumen promoting obstruction, or give rise to other pathological conditions. Meckel's diverticulum is only mentioned on account of its importance to surgeons (see Mixer 1933). Much literature is cited by Coled (1936).





FIG. 120. Mucosal lining of different regions of the digestive tract from a rabbit. The rabbit was killed by hanging. He refused all food during the last few days of his life. Much of lymphocytes have made their way into the submucosa. This is one of the most interesting histological modifications in starvation. The fundus of the stomach (A) the fundus of the stomach (B) the duodenum (C) the ileum (D). But the surface epithelium of the stomach and colon is much more heavily squamated in the duodenum and ileum.  $\times 100$

**Large Intestine.**—The gateway to the large intestine is the ileocecal valve—more accurately the *ileocecal sphincter*—just as the small intestine is guarded by the pyloric sphincter “. . . again there is a barrier, not only between the contents of two portions of the tract, but also between the peristaltic waves on the two sides, again this barrier between activities on the two sides is not complete; and again it appears to be due mainly to a folding of the muscle layers and an interposition of connective tissue” (Alvarez, 1929).



Contracted



Distended

FIG 131 —Effect of contraction and distention on structure of large intestine of the guinea-pig  $\times 80$  (Redrawn from Johnson, courtesy of Am Jour Anat.)

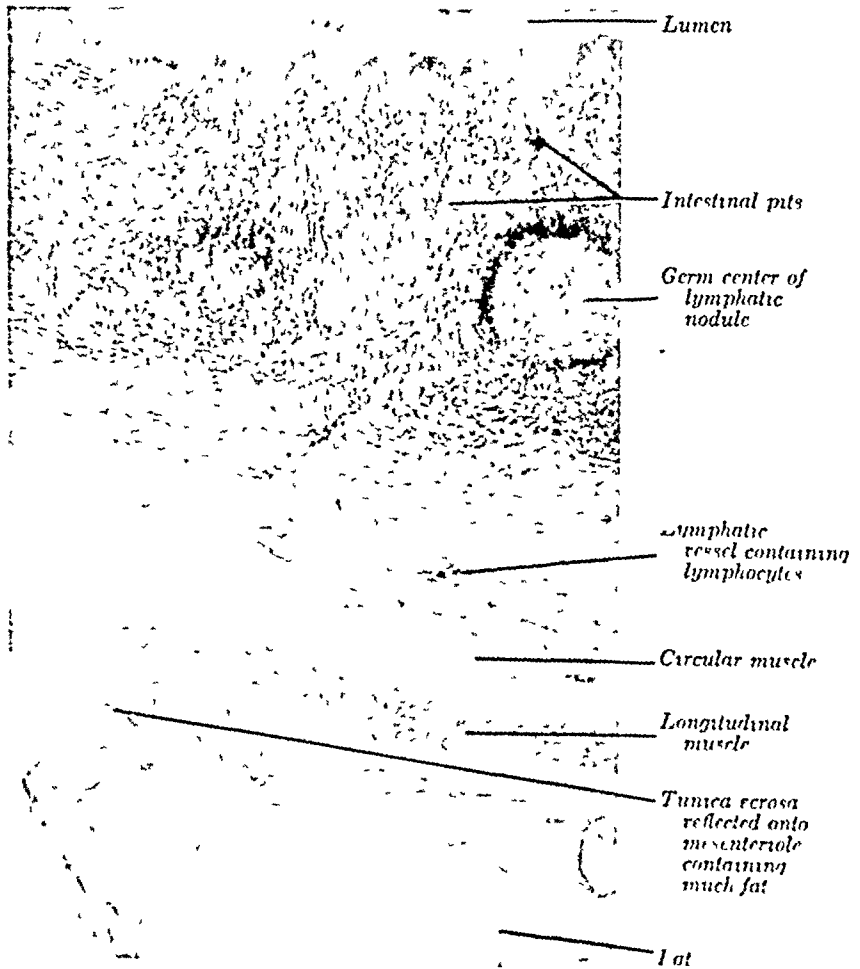


FIG. 132 Appendix, human Zenker-formalin H & E  $\times 70$

The plan of architecture is the same as in the stomach and small intestine—mucosa, muscularis mucosae, submucosa, muscularis and serosa—but, since the

functions served are not the same these basic components exhibit regional adaptations. Though the contents are rendered less fluid by removal of water, absorption is less and villi are dispensed with. Need for lubrication is increased and much mucus is produced which also plays a role in giving proper consistency to the feces. Movement onward is slow and hesitating. Material accumulates and the girth of the lumen is greater than that of the small intestine. Decomposition by bacterial action is a normal occurrence. Lymphatic tissue is abundant in the lamina propria especially where the contents stagnate.



FIG. 133—Colon human Zenker formalin H & E  $\times 70$

It will be recalled that there are *three* principal salivary glands. In the stomach *three* types of glands occur and in the small intestine *three* segments are recognizable: cecum, colon, and rectum. The appendix—a useless appendage always present—reminds us of Meckel's diverticulum of the small intestine—an unwanted complication rarely present but also sometimes productive of trouble for the owner. The appearance of the wall of the large intestine depends upon whether it is contracted or distended, but the expansion represented in figure 131 is greater than that commonly seen.

The *cecum* (*l. caecus*, blind) is a blind pouch which hangs downward in the abdomen below the level of the ileocecal sphincter. Its mucosa resembles that of the colon and rectum and will be described under colon. Its lumen is sacculi because the longitudinal muscle is exaggerated in three bands or *taeniae* (*G. lumen* a ribbon) which are shorter than the bowel would otherwise be. These bands converge to the point of origin of the appendix.

The *appendix* is a worm-like process (*vermiformis*) attached to the cecum. In the narrow lumen materials may lodge. The mucosa is similar to that of the colon except that there is always a concentration of lymphatic follicles in the lamina propria well developed even at birth. Paneth and enterochromaffin cells are

occasional findings. Care must be taken not to confuse the latter with macrophages containing phagocytized red blood cells, which give to their cytoplasm a yellow color. These macrophages differ from enterochromaffin cells by not being located within the epithelial layer and by the yellow material not being finely and uniformly particulate. Eosinophilic leucocytes are normally quite numerous. In old age the appendix shares in the lymphatic atrophy and its lumen may become occluded by fibrosis.

The *colon* is the longest of the three segments and the one most commonly painful (hence the word, *colic*). Note, in passing, that pain in the alimentary tract is chiefly occasioned by the stretching of inflamed tissue already swollen by infiltration of leucocytes and increase in tissue fluid (edema). The intestines can be burned and cut without conscious pain. The heroism of Japanese hari-kari is mostly limited to the anterior abdominal wall. Naked-eye inspection of the colon shows on the external surface the three *tæniæ* and fatty projections (appendices *epiploicæ*). The lumen is wider between the *tæniæ* and is segmented into saccular dilatations by *phlicæ semilunares*. The mucosa is quite smooth. On microscopic examination many pits are seen which extend straight in from the surface (Fig 133). Most of the epithelial cells are of the goblet variety but some are absorptive and possess thin striated cuticular borders. In the lamina propria are many lymphocytes and their plasma cell derivatives. Lymphatic follicles are not uncommon. The grouping of a large fraction of the longitudinal muscle into 3 longitudinal *tæniæ* has been mentioned. This is a conspicuous feature of transverse sections including one or more *tæniæ*. When the living colon, or any other part of the tract, is exposed to the air, or is rubbed, the muscles contract and contraction bands are apparent in stained sections. These are well shown in figure 134. When, on the contrary, the gut is fixed a considerable time after death, while in a completely relaxed state, the bands are not observed. Diverticulosis of the colon has a definite age incidence; weakening of muscle and elastic tissue is probably one factor. For the very important changes with age of this and other parts of the alimentary tract see Ivy (1942).

The *rectum* (*L. rectus*, straight) is a straighter part of the large intestine extending to the anus. Sacculations in the wall are absent; but there are three, or even four, transverse folds of the mucosa, supported by circular muscle, which tend to hold back the feces. The lower part, or anal canal, shows longitudinal folds (columns of Morgagni) and the epithelium becomes stratified. The inner sphincter is of smooth muscle; the outer one is composed of striated muscle—the first to occur in the tract since leaving the upper part of the esophagus.

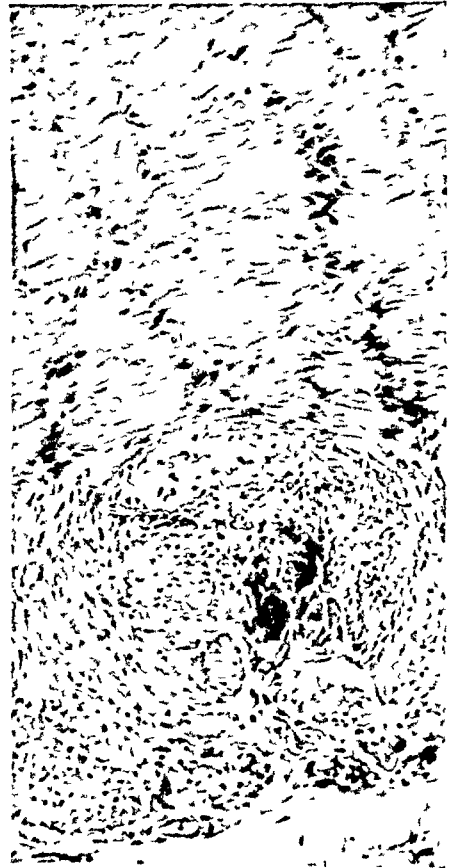


FIG 134—Contraction bands in smooth muscle of human colon. Zenker-formalin, H & E.  $\times 70$

This brief account has only included a few of the structural properties of the lower alimentary tract of the kind that the students can see in their preparations supplemented by a cursory examination of gross specimens. Data on nervous control are best assimilated in the courses on neuroanatomy and physiology in

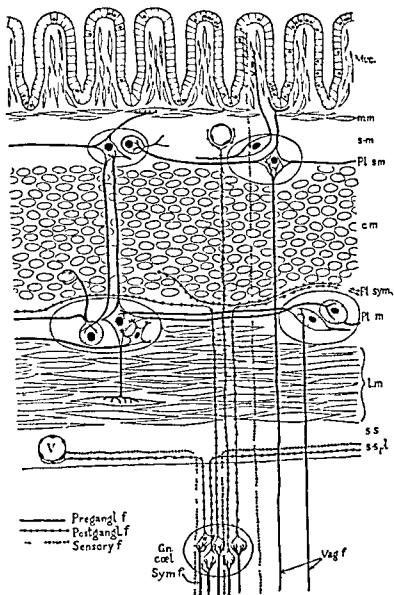


FIG 133.—Diagram of enteric plexuses. *cm* circular muscle. *Gn. cæl* celiac ganglion. *l m* longitudinal muscle. *m m*, muscularis mucosæ. *Muc* mucosa. *Pl m* plexus myentericus. *Pl s-m*, plexus submucosus. *Pl sym*, sympathetic plexus. *s-m* submucosa. *ss* serosa. *ss pl* subserous plexus. *Sym f* sympathetic fibers. *Vag f* vagal fibers. *V* vessel. (Redrawn from Hill Phil Trans Roy Soc London)

which they are linked with action. But the students should at least identify nerve cells in the submucous and myenteric plexuses (Fig 133) by review of all the slides. The blood supply is of the same general pattern throughout (Fig 129) with vessels parallel to the muscle fibers in the tunica muscularis branching profusely in the submucosa and proceeding vertically into the mucosa. This portion of the

histological course should be dynamically concluded with a second showing of the movie "Mesenteric lymphatics, their conduct and the behavior of their valves in the living rat" by Dr. Richard L. Webb of the University of Illinois, College of Medicine

### SUMMARY

The stomach is a large, mechanical mixer and secreter of digestive fluids. Entry through the esophagus, of food broken up and partly digested in the oral cavity, is guarded by the cardiac sphincter, which, however, accepts everything offered of a size able to pass in. By rather feeble contraction it prevents regurgitation, being helped by gravity, for fluids are not so easily pushed up hill as down dale. Exit is guarded by the much more effective and discriminating pyloric sphincter. This second sphincter is nicely regulated. Histologically and functionally it separates the stomach from the duodenum and enables each to perform its duties without interference. Because it allows the contents to pass to the duodenum only after proper preparation, which takes time, and food is taken much more quickly, the stomach also must act as a temporary storage mechanism. The epithelial lining consists of a single delicate layer of cells—very different from the resistant, stratified epithelium of the oral cavity, pharynx and esophagus—which is folded and invaginated to form crypts and glands. This produces mucus, pepsin, hydrochloric acid and much water for dilution. The muscular wall is built of three layers: inner oblique, middle circular and outer longitudinal. Waves of contraction churn up the contents. Motility is facilitated by an outer, moistened serous covering which minimizes friction on adjacent viscera. The stomach protects itself, and the body as a whole, against ingested pathogenic microorganisms, poisonous substances and mechanical injury by (1) its digestive secretions, particularly hydrochloric acid, (2) its protective mucus, which is increased enormously on irritation, (3) its capacity to regenerate new epithelium, and (4) its notable reluctance to absorb substances that might prove harmful. In addition to preparing the food for further digestion and absorption in the small intestine, the stomach bears the shock of concentrated substances swallowed by diluting them and inactivates or kills many invasive bacteria and parasites.

In the small intestine the digestion of food squirted in from the stomach continues by enzymes of intestinal origin (crepsin, invertase, etc.), supplemented by others from the pancreas (trypsin, amylase, lipase) and with the cooperation of bile from the liver, while special provision is made for absorption. The small intestine does not serve as a container like the stomach. Consequently its outlines are more even, despite the numerous coils, and its longitudinal and circular muscles are less developed. The contents are shuffled back and forth over the absorbing surface by rhythmic segmentation and are forced on by peristalsis. The outer surface, except for a part of the duodenum, is covered with a smooth friction-decreasing serous coat. The inner absorbing surface is ridged and lined with a mucous membrane pitted by intestinal crypts which, unlike the gastric ones, end blindly and do not serve as ducts for highly differentiated glands. Between these crypts the epithelium extends into the lumen, forming long delicate villi that greatly increase the surface. Most of the epithelial cells possess a typical distal striated border not found in the stomach. Goblet cells come next in order of frequency, followed by the much less numerous Paneth and enterochromaffin cells. In the duodenum glands of Brunner occur between the muscularis mucosae and the tunica muscularis. No secretion

## LOWER ALIMENTARY TRACT

antecedents have been found for the intestinal enzymes mentioned or for secret a cholecystokinin or any other hormone. Hormonal regulation is more highly developed in the duodenum than in any other segment of the digestive tract perhaps partly on account of the size and importance of the accessory glands (liver and pancreas) and proximity of the pylorus. The small intestine as a whole also exhibits clearly defined axial gradients. Replacement of epithelial cells takes place especially from the crypt epithelium, but it is less noticeable than in the stomach through which the ingested material must first pass. We have referred to the histological margin of safety (p. 229). There is also a related duplication of enzymes. The salivary glands and pancreas produce starch splitting enzymes while the stomach and pancreas form proteolytic enzymes and lipases. The utilization of food is therefore a sustained process. A functional deficiency in a particular enzyme at one level in the digestive tract is compensated for, at least partly, by the supply of the same enzyme in another.

The large intestine, like the small one and the stomach, is shut off by sphincters above and below. Normally it stores the contents longer and absorption of water is a specialty. Villi are absent. Only absorptive epithelial cells with striated border and goblet cells are present. No digestive enzymes are required but bacterial decomposition normally occurs. With increase in consistency of contents more protective mucus is needed and the peristaltic contractions are slow but forceful. The contents remain in touch with the wall for a considerable time particularly in the cul-de-sac appendix and colon part of the cecum and rectum being usually empty. The relative amount of subepithelial lymphoid tissue decreases in this sequence. Except for contact stimulation of the stomach, no hormones are produced. As in the stretches of the tract above the stomach, no details of the process have not been worked out.

In reviewing the whole tract the following points may be emphasized: (1) The voluntary control of the two ends and the subconscious action of the intercurrent part under intrinsic nervous and hormonal regulation. (2) The protection given at the entrance which is not required at the exit. (3) The special hormonal integration developed in the chief sites of digestion the stomach and the duodenum for it is into the latter that the pancreas and liver pour their secretions. (4) The suspension of the stomach all of the small intestine except the duodenum and most of the large intestine by smooth sheets of peritoneum (the mesentery) in large slippery serous cavity comparable to the pericardial sac thus decreasing friction and facilitating movement which otherwise would be impossible. (5) The increase in epithelial surface for the absorption of products of digestion in the small intestine by the formation of villi and by folding of the mucous lining. (6) The absorption of water concentration and temporary storage of the residue in the large intestine.

## CHAPTER XI

### GLANDULAR APPENDAGES

**Pancreas.**—This is like a huge, misplaced salivary gland which has learned how to produce the secretions required in the segment of the digestive tract immediately following the stomach, and which, in addition, functions as an endocrine. Examined in the fresh state the pancreas is white tinged faintly red by the blood in it. In consistency it resembles the loosely-knit sublingual rather than the firm parotid. The thin investing layer of connective tissue does not form a definite capsule. By



FIG. 136 —Islands of Langerhans of a guinea-pig, stained by injection of neutral red into the blood vessels, showing variations in the islets and the general appearance of material used for their enumeration. (From Opie, after Bensley, *Special Cytology*, Paul B. Hoeber, Inc.)

penetrating between the lobules and groups of secretory cells (acini) it affords pathways for blood vessels, lymphatics and nerves. The larger lobules are visible without magnification. Because they are thin and can be separated so easily, the gland is well adapted for microscopic study.

In the first place it is necessary clearly to distinguish the exocrine and endocrine components. Small pieces of a fresh pancreas which has been colored supravitaly with neutral red by Bensley's (1911) method are used.

Add 2 cc. of a previously prepared 1 per cent solution of neutral red in distilled water to 300 cc. of physiological salt solution thus making a concentration of neutral red of 1 to 15,000. Place this, and as much more as may be required, in a bottle from the bottom of which a glass tube leads off or in an ordinary bottle with a bent glass tube serving as a siphon. The tube is to be connected with a cannula by about 6 feet of rubber tubing. A freshly



## GLANDULAR APPENDAGES

killed guinea pig is bled from the throat. Insert the cannula in the thoracic aorta and let the solution by raising the bottle to a height of 4 or 5 feet. Lay bare the pancreas. Cut the inferior vena cava near the heart so that the blood in the vessels followed by the solution may easily escape. When the injection has reached the proper stage the pancreas will have assumed a deeper rose color. Remove small pieces, mount in physiological salt solution under cover glasses and examine at low magnification.

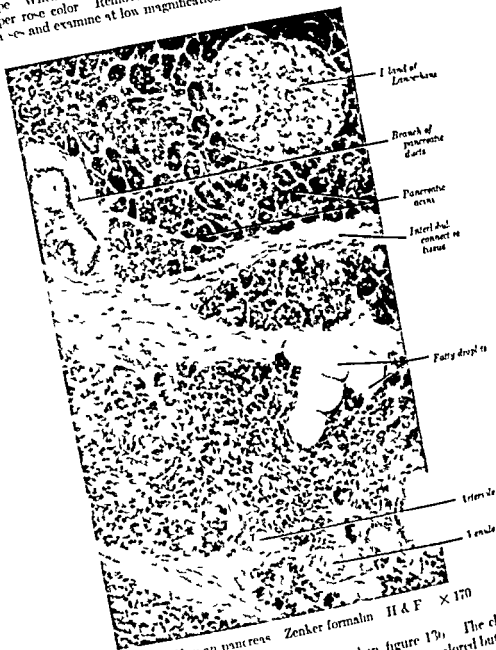


Fig. 135. Human pancreas. Zenker formalin. H & F.  $\times 170$

A preparation made in this way is illustrated in figure 135. The clumps of exocrine cells which constitute most of the glands are colored but should be stained with fast blue. The endocrine cells which produce the internal secretion are stained with fast blue. If the preparation is a little overexposed it could be examined more rapidly from the connective tissue than from the acini.

In ultraviolet light the islets of Langerhans of the human pancreas exhibit a yellow brown fluorescence of sufficient intensity sharply to distinguish them from the surrounding tissue (Grafflin, 1940) Fluorescence microscopy has come to stay.

In routine preparations the islets do not stand out so sharply Section of a large islet is included in figure 137 Its outline is rounded, its substance is not broken up into acini, like those of the surrounding tissue, and it is but faintly stained Close examination of almost any section of the pancreas shows islets of many sizes Practice is required in their identification After one minute of study, one may be found, after fifteen minutes a great many more are apparent They do not contain the large granules of secretion antecedent (zymogen) typical of the acinous cells

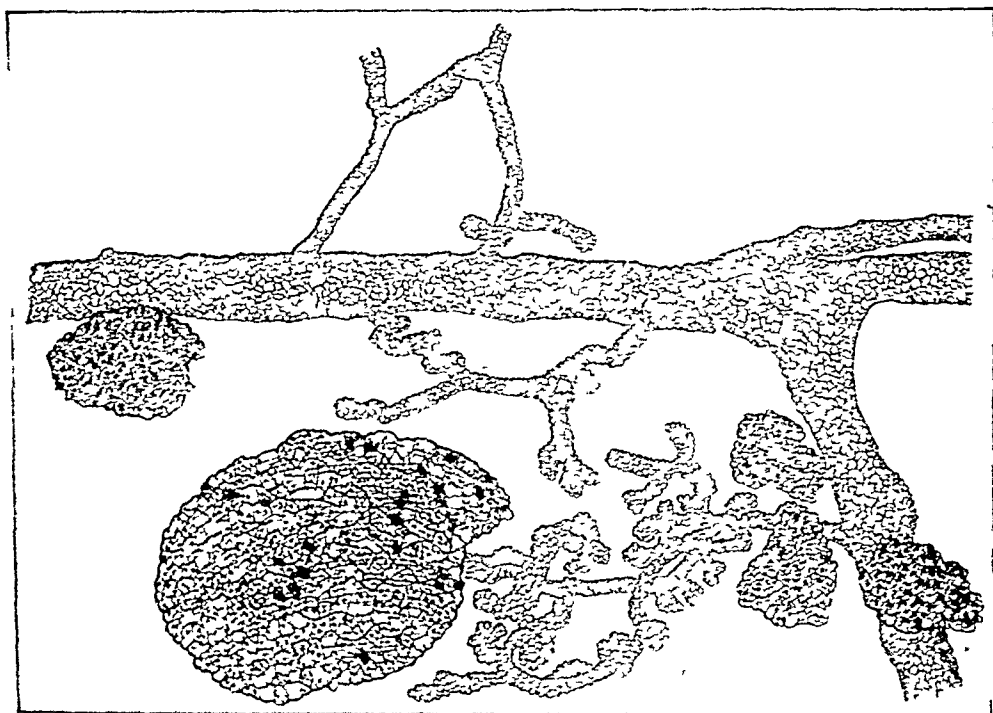


FIG 138 —Highly branched tubules connecting a duct with an islet in the guinea-pig's pancreas. (Opie, after Bensley, Special Cytology, Paul B Hoeber, Inc)

Ducts of two kinds are present: those that carry secretion and those that do not The first are, when large, obvious structures easily identifiable by their simple epithelial lining backed by connective tissue; when small they look rather like the intercalated ducts of the salivary glands (Fig 108) The second are extremely difficult to see in sections but can be demonstrated by supravital staining with pyronin as described by Bensley They are very delicate cords of cells, devoid of lumina, which connect the islets with ducts of the first type, the smaller ones of which stain similarly These branching ductules pervade the pancreas and constitute a comparatively undifferentiated tissue from which islets spring in normal development and in regeneration after injury.

The islets appear to have a high priority rating In a supravitaly stained pancreas they are often rendered visible merely by the close networks of capillaries within them in comparison with the fewer capillaries of the acinous tissue Beck and Berg (1931) have expressed the view that they are very directly supplied with

# GLANDULAR APPENDAGES

178

killed guinea pig is bled from the throat. Insert the cannula in the thoracic aorta and use the solution by raising the bottle to a height of 4 or 5 feet. Lay bare the pancreas. Cut the inferior vena cava near the heart so that the blood in the vessels followed by the solution may easily escape. When the injection has reached the proper stage the pancreas will have assumed a deeper rose color. Remove small pieces, mount in physiological salt solution under cover glasses and examine at low magnification.

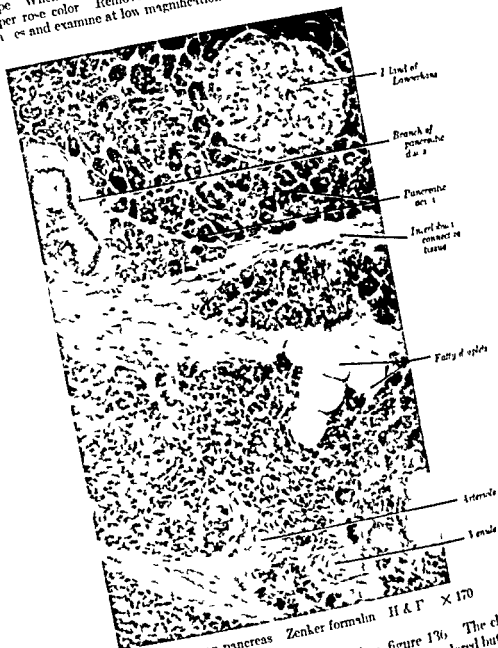


Fig. 147 Human pancreas Zenker formol H & E  $\times 170$

A preparation made in this way is illustrated in figure 136. The clumps of exocrine cells or acini which constitute most of the glands are colored but little, whereas the cells of the islets which produce the internal secretion are stained intensely. If the preparation is a little over-stained it should be set aside for half an hour and re-examined. The contrast may thus be increased because the islets often take more rapidly from the acinous than from the islet tissue.

In ultraviolet light the islets of Langerhans of the human pancreas exhibit a yellow brown fluorescence of sufficient intensity sharply to distinguish them from the surrounding tissue (Grafflin, 1940) Fluorescence microscopy has come to stay.

In routine preparations the islets do not stand out so sharply Section of a large islet is included in figure 137 Its outline is rounded, its substance is not broken up into acini, like those of the surrounding tissue, and it is but faintly stained Close examination of almost any section of the pancreas shows islets of many sizes Practice is required in their identification After one minute of study, one may be found, after fifteen minutes a great many more are apparent They do not contain the large granules of secretion antecedent (zymogen) typical of the acinous cells

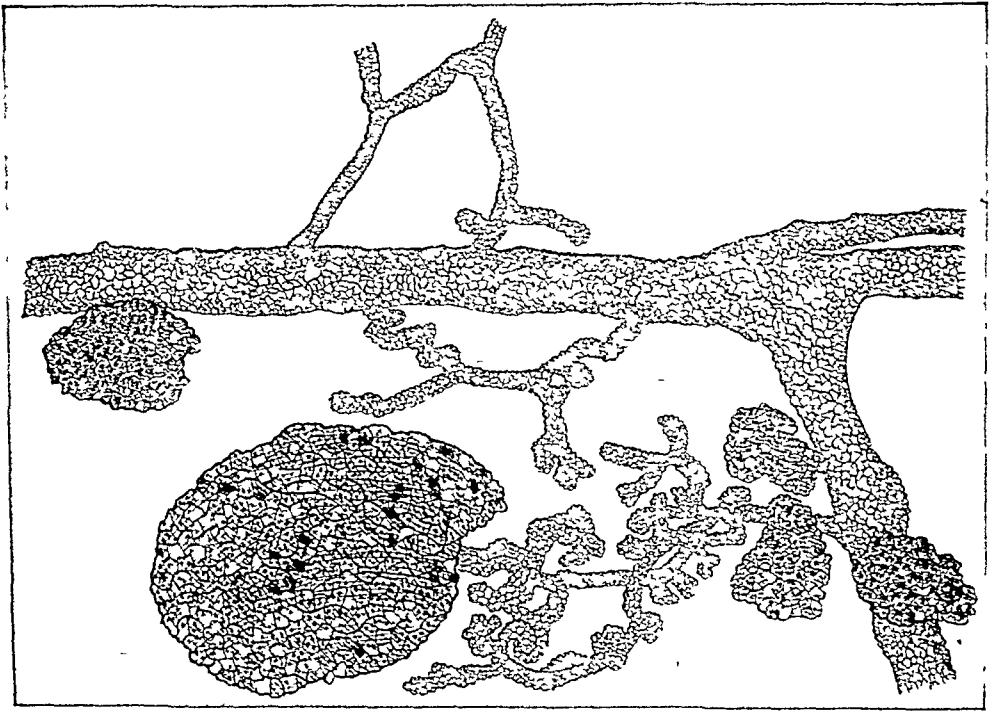


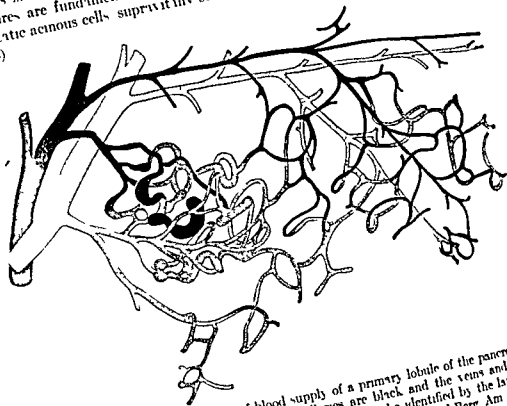
FIG 138 —Highly branched tubules connecting a duct with an islet in the guinea-pig's pancreas (Opie, after Bensley, *Special Cytology*, Paul B Hoeber, Inc )

Ducts of two kinds are present those that carry secretion and those that do not The first are, when large, obvious structures easily identifiable by their simple epithelial lining backed by connective tissue, when small they look rather like the intercalated ducts of the salivary glands (Fig 108) The second are extremely difficult to see in sections but can be demonstrated by supravital staining with pyronin as described by Bensley They are very delicate cords of cells, devoid of lumina, which connect the islets with ducts of the first type, the smaller ones of which stain similarly These branching ductules pervade the pancreas and constitute a comparatively undifferentiated tissue from which islets spring in normal development and in regeneration after injury

The islets appear to have a high priority rating. In a supravital stained pancreas they are often rendered visible merely by the close networks of capillaries within them in comparison with the fewer capillaries of the acinous tissue Beck and Berg (1931) have expressed the view that they are very directly supplied with

The structure and mode of secretion of cells under intensive investigation for many years. Mention will be made of the technique of O'Leary (1930) and of the structure of the plasma membrane. The structure and mode of secretion of cells under intensive investigation for many years. Mention will be made of the technique of O'Leary (1930) and of the structure of the plasma membrane.

1 It is a waste of time like a parrot to repeat with every cell encountered that it contains *mitochondria* of course it does if the cell is alive and well. But these structures are fundamental cellular constituents and they can best be seen in pancreatic acinous cells, supravitaly stained with junos green and in lymphocytes (p. 20)



The schematic drawing of blood supply of a primary lobule of the pancreas of a white mouse. The arteries and arterial capillaries are black and the veins and venous capillaries are gray. The kind of Langerhans can easily be identified by the large grist and pleomorphic arrangement of its capillaries. (Redrawn from Beck and Berg, *Am. J. Path.*)

The best way to color the entire pancreas of a guinea pig by injecting through the porta  
a 1 to 10,000 solution of the dye in physiological saline solution as described by Herber  
(1911). Tiny pieces are then removed and mounted in the same solution for study. They  
must be small that the pressure of the cover glass alone is sufficient to flatten them out.  
Otherwise, even with the use of the superposition of many cells. If there is enough  
fluid to float the cells, it should be removed from the edges by filter paper. The  
distal part of the pancreas is marked by large numbers of highly refractile uncolored symen-  
granules. It is in the proximal parts that the mitochondria will be observed colored blue-  
green. They are like fat granules in like shape is that distinctive (Fig. 140 B). Some can even be  
made out in the white granules. It is taken the stain (A). Their shape and distribution in the  
central part of the pancreas is different. The dye is slowly reduced in the  
proximal part of the pancreas to a pink colored substance and then to a colorless liquid. The  
green color is lost (Fig. 140 C). (Apply of atmospheric oxygen the mitochondria retain the  
green color.) (Fig. 140 D) (p. 101)

It will be observed that, while the length of mitochondria is variable, their diameter is astonishingly uniform. The total mitochondria cytoplasmic surface is considerable and can be measured (DuNouy and Cowdry, 1927). In an epoch-

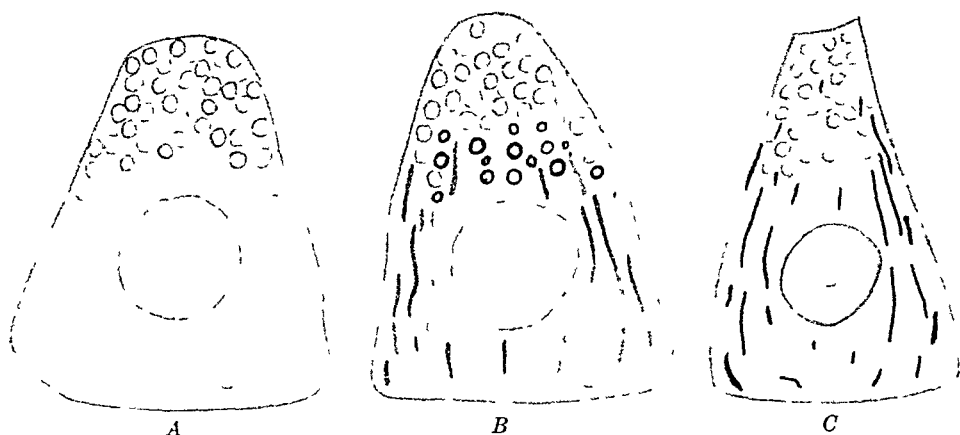


FIG 140 —Diagram of appearance of pancreatic acinous cells of mice. A, Viewed in the living state unstained, B, after supravital coloration with janus green and neutral red, C, following fixation in Regaud's fluid and staining with fuchsin and methyl green. Zymogen granules appear in the distal ends of the cells in all three. Mitochondria are barely visible in A, represented in gray in B, and black in C. A spherical fat droplet is illustrated in the proximal parts of A and B. The black circles in B represent neutral red granules. Optically homogeneous chromidial substance is indicated by the darker shade of gray in the proximal zone of C.

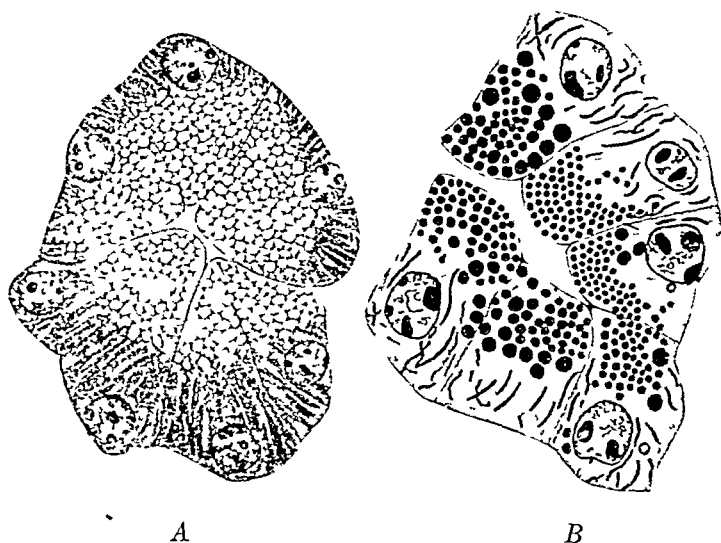


FIG 141 On left section of pancreatic acinus of guinea-pig. Chromidial substance in proximal zone appears dark having been stained with toluidin blue with light spaces representing original location of mitochondria. Zymogen granules in distal zone appear as spherical unstained spaces. On the right an acinus stained with fuchsin and methyl green. Here mitochondria appear as black filaments and zymogen granules as black spherules. (From Opie in Cowdry's Special Cytology, courtesy of Paul B Hoeber, Inc.)

making cytological study Bensley and Hoerr (1934) separated liver cell mitochondria from other parts of the cell and subjected them to direct chemical analysis. They found two proteins and an average of 43.6 per cent of fatty substances soluble in hot alcohol, ether and chloroform. Probably these acinous cell mitochondria

are likewise rich in fats. Theories on the role of mitochondria in vital processes are summarized by Bourne (1912)

2. In the intermediate zone of cytoplasm a few droplets stainable with neutral red (*neutral red granules*) can be identified in these (Fig. 140, B) as well as in a wide variety of other kinds of cells.

3. Other components require fixation and special techniques for their demonstration. Use of a basic dye like toluidin blue shows in the proximal cytoplasm a homogeneous looking deposit of *chromidial substance* (Fig. 141, A). It colors (chroma color) like basophilic nuclear chromatin and in fact is said to be of nuclear origin. In such preparations only the outlines of the zymogen granules are distinguishable. In the chromidial substances the spaces occupied by the mitochondria

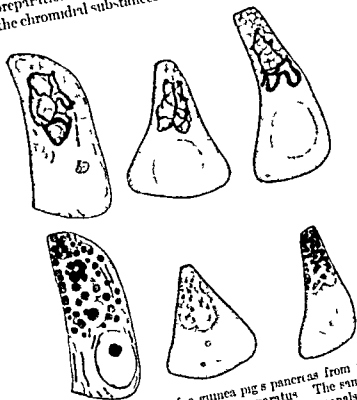


FIG. 141. Three zymous cells of a guinea pig's pancreas from three specimens each blackened with osmic acid to reveal the Golgi apparatus. The same cells after bleaching and staining with iron hematoxylin show systems of clear canals corresponding exactly with the thickened networks. (Cowdry, *General Cytology*, University of Chicago Press.)

look empty. Coloration with fuchsin and methyl green (B) shows zymogen granules and mitochondria in crimson (black in the figure) and the chromidial substance as a homogeneous green material in the spaces between the nerve cells. This chromidial substance is related to the Nissl bodies of nerve cells. It is also found in many other zymogenic or enzyme-producing cells.

4. By impregnation with silver or osmium it is generally feasible after consideration of clear canals and their position. The negative of this apparatus—between the nucleus and the Golgi apparatus—rarely encountered in impregnated tissue because the two have the same shape and position.

Structural details of these components with different stages in pancreatic secretion have been described again and again. Both Golgi apparatus and mito-

chondria show changes (Fig 143) but their contribution to the formation of zymogen granules is not known. The ground substance may play the star rôle. Recent studies on the submicroscopic structure of acinous cells may turn out to be important. Examination of a beautiful colored plate depicting observations by Ries (1940) is at least in order. Covell's (1928) continuous observation of the process of secretion *in vivo* should be extended. He found that the fate of neutral red granules

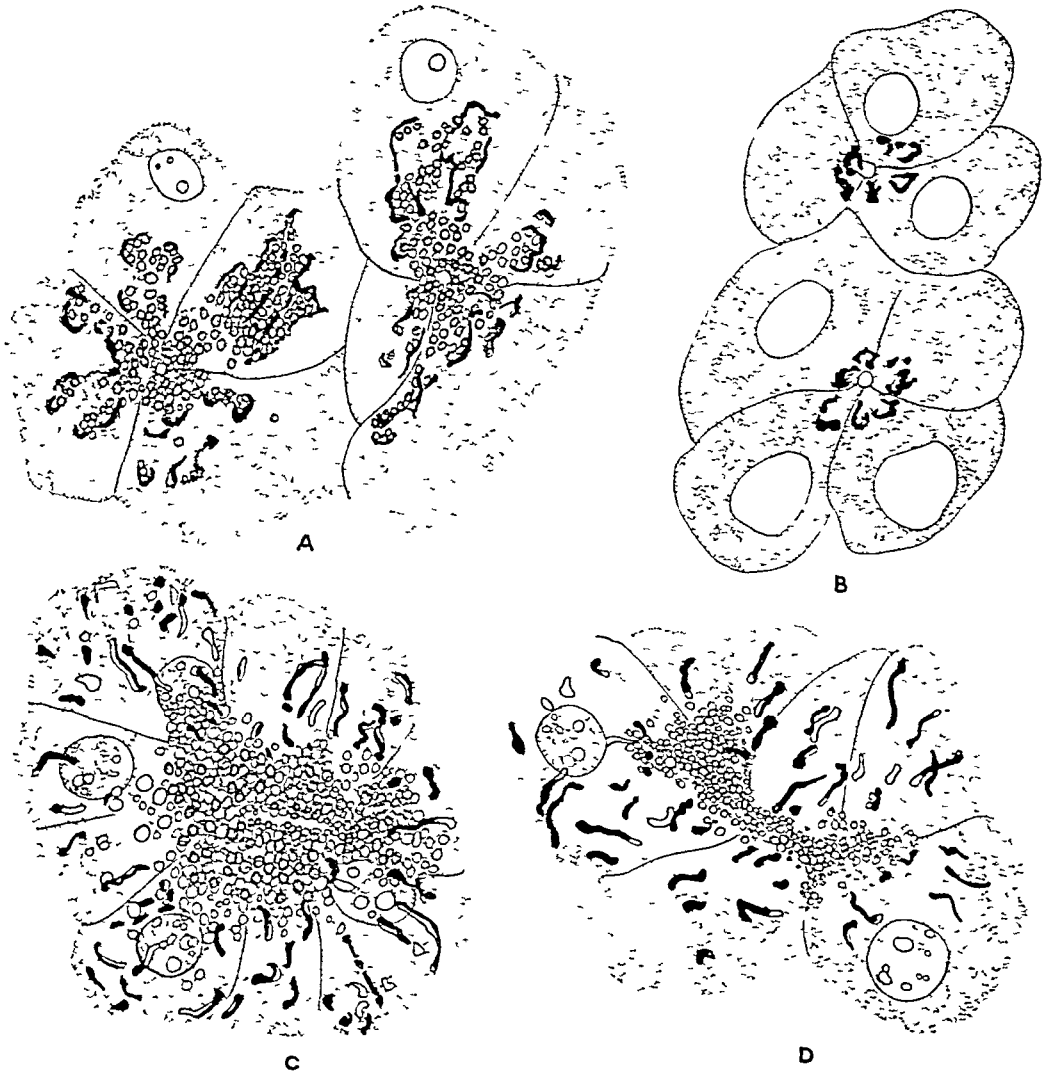


FIG 143 —Effect of pilocarpine on the Golgi apparatus and mitochondria of pancreas of white mice. *A*, Golgi preparation of normal pancreas, *B*, three hours after injection. Disappearance of zymogen granules and movement of the blackened Golgi apparatus toward the lumen. *C*, Mitochondrial preparation of normal pancreas, *D*, three hours after injection. Decrease in zymogen granules and mitochondria. (Nassonov, Arch f mikr. Anat.)

can be followed if they are highly colored with neutral red (Fig 144). The zymogen granules are easily distinguishable by their high refractive index. The significant fact, which Covell brought forward, is that there is an outpouching of the cell membrane into the lumen. These pouches presumably contain secretion antecedents. They are pinched off and escape into the lumen, so that cellular material leaves the cell without passing through the cell membrane. A similar phenomenon was later observed by de Robertis in the thyroid (Fig 74).



It is instructive to demonstrate at this time a moving picture film in the War Institute series by Dr. W. H. Lewis entitled "Pinocytosis—drinking by cells" which shows the reverse process of intake of fluids by invagination of the cell membrane and its subsequent elimination with liberation of the fluids in the cytoplasm without diffusion through an intact cell membrane.

The figures 143 and 144 show the discharge of secretion under the influence of pilocarpine. Atropine would bring about retention. There are two normal stimulants of secretion—vagal impulses that call forth a fluid rich in enzymes and the hormone secretin, which brings about an outpouring of water and mineral constituents (Best and Taylor, 1939). The enzymes are activated by intestinal

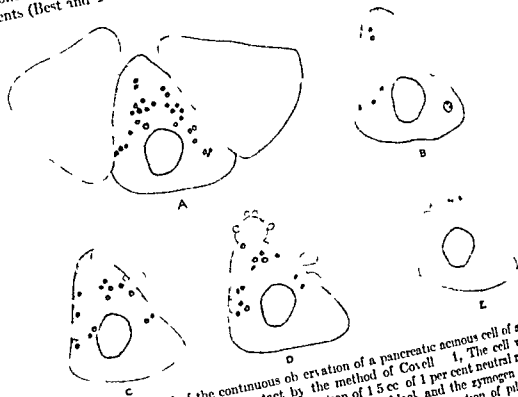


FIG. 144—Record of the continuous observation of a pancreatic acinous cell of a white mouse observed with circulation intact by the method of Covell. A, The cell with its neighbors outlined forty minutes after the injection of 1.5 cc of 1 percent neutral red dye. The granules stained by the dye are indicated in black and the zymogen granules are represented by gray circles. B, Same seven minutes after injection of pilocarpine. C, Same ten minutes later. Note appearance of secretion vacuoles which with the zymogen granules are leaving the cell. D, Same twenty five minutes after pilocarpinization increased exodus of vacuoles and granules. E, Same fifteen minutes later. Cell discharged except for a small vacuole on the left. The size of the cell is reduced as compared with A. The neutral red granules are less noticeable and have shifted toward the lumen and the zymogen is reduced. X 1,000. (Redrawn from colored figures Covell Anat. Rec.)

okinase. That the acinous cells of a single type should produce even enzymes (trypsin, amylase, lipase, rennin, maltase, glyoxal, glycine dipeptidase, and carboxy polypeptidase) and perhaps an eighth (erepsin) justifies for them the crown of the most talented zymogenic cells of the body. Acidophilic and basophilic cells of the alimentary tract taken together produce about as many hormones.

There is a fine opportunity in both organs for the correlation of cytological and humoral secretions—line of activity which is rejuvenating the old science of

microscopic anatomy In the pituitary the cytoplasmic granules are expressions of distinctive cellular organization, not actual secretion antecedents like the zymogen granules of the pancreas The zymogen granules are of variable size, but, to our rather crude tests, they appear to be of uniform composition Until satisfactory evidence is discovered of the existence of several types of granule, the assumption is justified that each granule contains several enzymes, or proenzymes, later to be activated Investigations by Van Weel and Engel (1938) and by Van Weel (1939) indicate that dipeptidase is not present in the granules, which ordinarily are cast out, but in the small amounts of associated cytoplasm which accompany them when an unusually strong stimulus to secretion is applied Apparently the dipeptidase takes part in the production of carboxylpolypeptidase, the amount of which is proportional to the number of granules

As the cellular machinery wears out in old age all of the enzymes produced by the acinous cells do not fail in the same degree because Meyer *et al* (1940) have discovered that lipolytic and amylolytic activity is but slightly depressed whereas proteolytic activity is decidedly reduced Apparently in the same cell the synthetic processes of enzyme building, whatever they may be, are to some extent independent and subject to individual modification

It is interesting to stop to consider in what cells of the digestive tract, besides the acinous cells of the pancreas, these enzymes are to be found Cells of the villi are discounted because they are absorptive and liable to pick up enzymes of pancreatic origin discharged into the intestinal lumen *Amylase* is present in the salivary glands and Brunner's glands of rabbits, *lipase* in the cardia, fundus and pylorus of the stomach of pigs, *rennin* in the chief cells of gastric glands of the fundus and *dipeptidase* in peptic cells of gastric glands of fundus, in deep cells of pyloric glands and in Brunner's glands Evidently pancreatic acinous cells enjoy the ability of producing enzymes shared by other cells some of which also form mucus

Little wonder that the job of producing insulin is assigned to other than these hard worked acinous cells In hematoxylin and eosin stained sections it may be difficult to distinguish the types of cells present in the *islets of Langerhans* but by the use of special stains two types of cells can be surely identified The A cells are often first in the sense that they are prone to occur at the periphery of the islets and are first encountered as the eye travels from the surrounding acinous tissue into the islet The B cells are normally by far the most numerous and contain granules possessed of distinctive staining properties which are soluble in alcohol—a significant point, since alcoholic extracts of pancreas contain insulin Other different looking cells occur in small numbers (indifferent cells, D cells, etc). Gomori (1941) suggests that the D cells represent but a stage in ageing of A cells

Evidence from tumors, so helpful in tracing hormones to their cells of origin in other endocrines, does not help much with the islets Though often accompanied by symptoms of hyperinsulinism, to classify the cells in them as definitely A or B may not be feasible (O'Leary and Womack, 1934). O'Leary (1930), closely observing the islet cells in the living pancreas, detected the formation of droplets in the cells in response to dextrose injections, and their movement toward the adjacent capillary, as well as their later decrease in size, presumably owing to diffusion of their contents through the cell membrane This is probably the mechanism of insulin secretion and the cells are in all likelihood of the B type but they could not be positively identified as such in the living state Several observations strongly suggest that B cells are in fact the chief producers of insulin (1) when, in dogs, a



large part of the pancreas is surgically removed a strain is placed upon the islets and it is the B cells (not the A cells) that undergo a watery degeneration, called hydropic, and eventually disappear coincident with the appearance of symptoms of diabetes mellitus (Homans, 1915, Allen, 1922), (2) repeated injections of insulin in rats produce changes indicative of suppression of activity of B cells without any alteration in A cells (Latta and Harvey, 1942), (3) injections of anterior pituitary extract cause in dogs a reduction in insulin content of the pancreas accompanied by a degranulation and hydropic degeneration of B cells. Daily administration of protamine zinc insulin, along with the pituitary extract, tends to prevent this reduction in insulin content and loss of B cell granules (Best, *et al*, 1942).

Looking backward, we find that diabetes was known to the Romans but it remained for a humble laboratory attendant to make an observation that directed attention to the pancreas some fifty-one years ago. It is related that the individual, while assisting Minkowski and von Mering in their studies on the rôle of the pancreas in digestion, "noted that the urine of dogs that were suffering from deprivation of the pancreas was attracting great swarms of flies. This curious happening was called to the attention of one of the investigators. Whereupon a chemical test of the urine was made. It was found to be loaded with sugar. Thus experimental diabetes had unwittingly been produced and the problem was now open to intensive investigation" (Hoskins, 1933). This demonstration that diabetes could be produced by removal of all or most of the pancreas led to the feeding of extracts to diabetics and "sweetbreads," already a delicacy, became the order of the day. Then followed a period of hopes and disappointments. Some investigators came within an ace of making strong preparations of the hormone which they knew existed and which was called "insulin." Banting, like Scott before him, had the idea that the digestive enzyme trypsin, poured by the pancreas into the duodenum, digested the hormone in the making of extracts. The trick was the elimination of trypsin. Banting and Best first made extracts of pancreases the ducts of which had been ligated, since it was known that this procedure caused atrophy of the trypsin secreting acinous tissue without interfering with the islets of Langerhans, which for many reasons were regarded as the endocrine component. They were successful and later, with the aid of Collip, made potent extracts from whole glands guarding against the destructive action of trypsin by chemical means. The story of this achievement, related simply by Hoskins and in detail by MacLeod (1924) in whose laboratory the work was done, affords entertaining reading. Insulin is one of the fundamentally most necessary of hormones. Our chief source of energy is carbohydrate food. The amount offered to the cells by the blood stream is regulated by insulin through storage as glucose, principally in the liver, and liberation from storage.

Before leaving the pancreas reference must be made to a second pancreatic hormone, the lipocalc of Dragstedt. The cytological source of this substance is not known. Deficiency in it appears to cause a loss in body fat and an accumulation of fat in the liver (Julian *et al*, 1943).

**Liver.**—Like the pancreas, the liver is developed from an out-pouching of the endoderm of the gut and throughout life it discharges into the duodenum as the pancreas does. But its primitive architecture as a compound tubular gland is obscured by the acquisition of a double blood supply without knowledge of which its structure never can be understood.

Observe in an animal under ether anesthesia that blood enters the liver not only by the hepatic artery but also by the portal vein. Note the area drained by the latter. Kill the animal by opening the peritoneal cavity and leaving the liver by the hepatic vein.

Examine the surface of the liver with a hand lens. Kill the animal by opening the peritoneal cavity and leaving the liver by the hepatic vein. Does the size or color of the liver change? Perfuse with 0.5% aqueous sodium chloride as described in Microscopic Technique (p. 153). Since the blood is displaced by colorless fluid first at the periphery of the lobules and last at their centers it should be possible to visualize the lobules as small red masses with lighter colored margins. Continue the perfusion till most of the blood is washed out. The yellow color of myriads of liver cells is unimpaired by removal of blood. Increase the perfusion pressure by clamping the vena cava and increasing the height of the perfusion bottle and observe how greatly the liver swells. Remove a little tissue and examine microscopically.

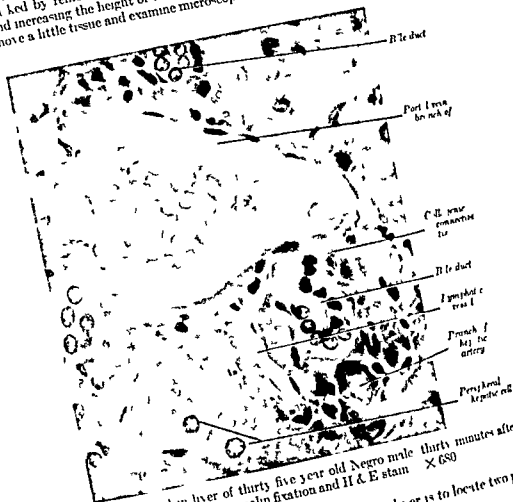


FIG. 147. Portal canal in liver of thirty five year old Negro male thirty minutes after excision. Zenker formalin fixation and H & E stain.  $\times 680$

The first aim in any microscopic examination of the liver is to locate two principal landmarks—the portal canals and the central veins. Portal canals are strands of connective tissue sandwiched between the lobules carrying branches of the portal vein, hepatic artery, bile duct and lymphatics. Since they usually are placed along the surface of contact between three lobules they have a somewhat triangular shape when viewed in cross sections which have been selected for study because they contain all of the structures mentioned. Where only some of them may be represented if the plane of section merely includes a depth through part of the length of a canal.

Figure 147 illustrates a typical portal canal. The collagenic fibers of the con-

With these data, the study will show that the method of determining the number of chromosomes in a cell is not only a simple and rapid method, but also a method which can be used for the study of the chromosomes of a cell in a very short time. The method is simple and rapid, and it can be used for the study of the chromosomes of a cell in a very short time. The method is simple and rapid, and it can be used for the study of the chromosomes of a cell in a very short time.

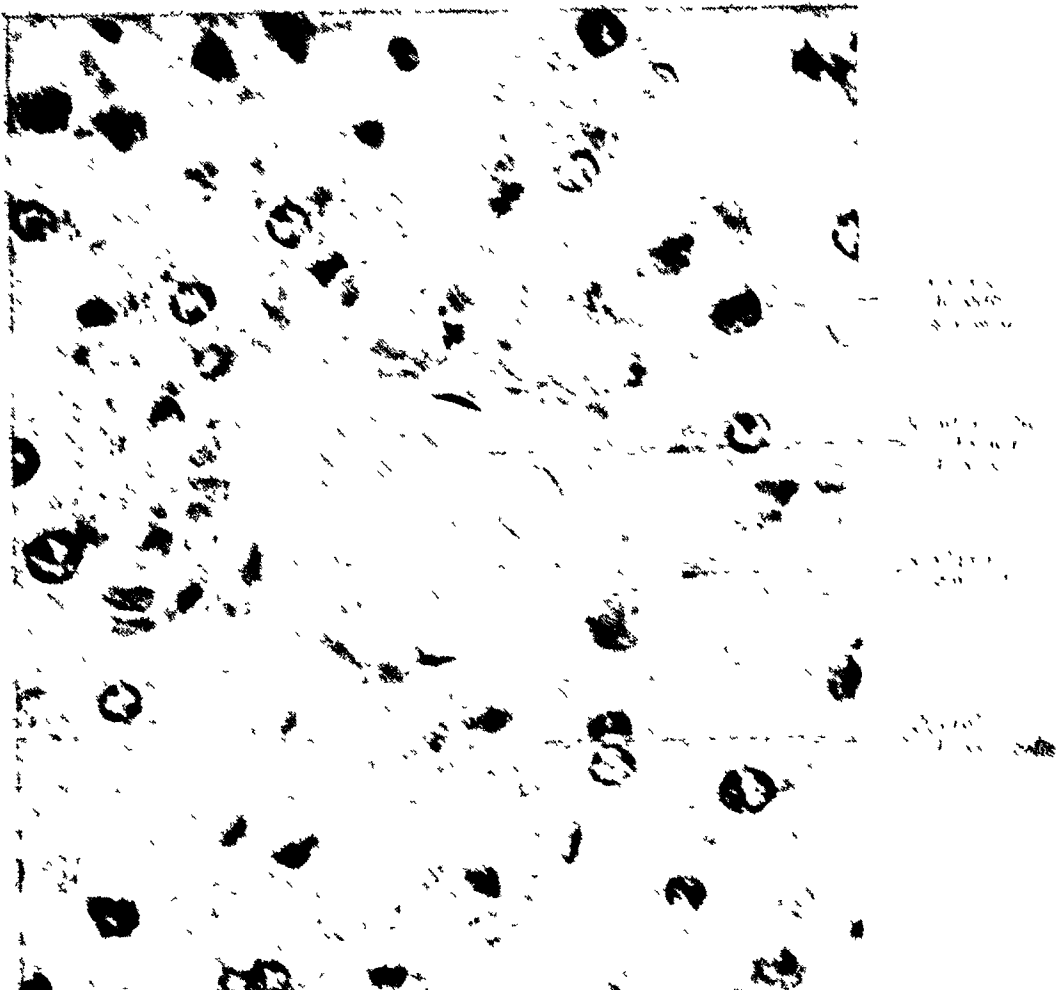


Fig. 1. Chromosomes of a cell in a very short time.

The method of determining the number of chromosomes in a cell is not only a simple and rapid method, but also a method which can be used for the study of the chromosomes of a cell in a very short time. The method is simple and rapid, and it can be used for the study of the chromosomes of a cell in a very short time.

Many have used the method of determining the number of chromosomes in a cell in a very short time. The method is simple and rapid, and it can be used for the study of the chromosomes of a cell in a very short time. The method is simple and rapid, and it can be used for the study of the chromosomes of a cell in a very short time.

100

GLANDULAR APPENDAGES

served by the vessels in several portal canals. Hepatic sinusoids carry blood received from the portal vein and hepatic artery into the substance of the lobule between cords of liver cells and empty into the central vein. Near the periphery of the lobule Wakim and Mann (1942) in their study of blood flow in rats were able to distinguish between sinusoids receiving arterial and venous blood but this was

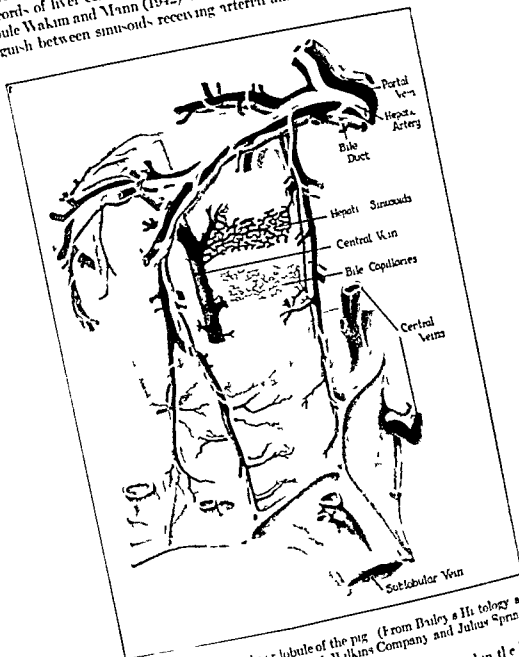


FIG. 149. B. Anat. (From Bailey & H. H. Company and Julius Springer)

net is supplied by the central vein (Fig 140). Circulation within the lobules was  
 irregular but constant. Occasionally the blood becomes stationary in the  
 lobules or even in the sinusoidal bed as well as the capillary bed.  
 much of the blood is in full activity. In the liver the factor of safety is a large  
 one. The direct blood flow in the cortex is likewise from out side in to

the composition of the blood is different since it is wholly arterial and not mainly venous. In both, the most favored cells are those first served near the periphery. These are the ones most capable of multiplication to make good a loss. Hepatic

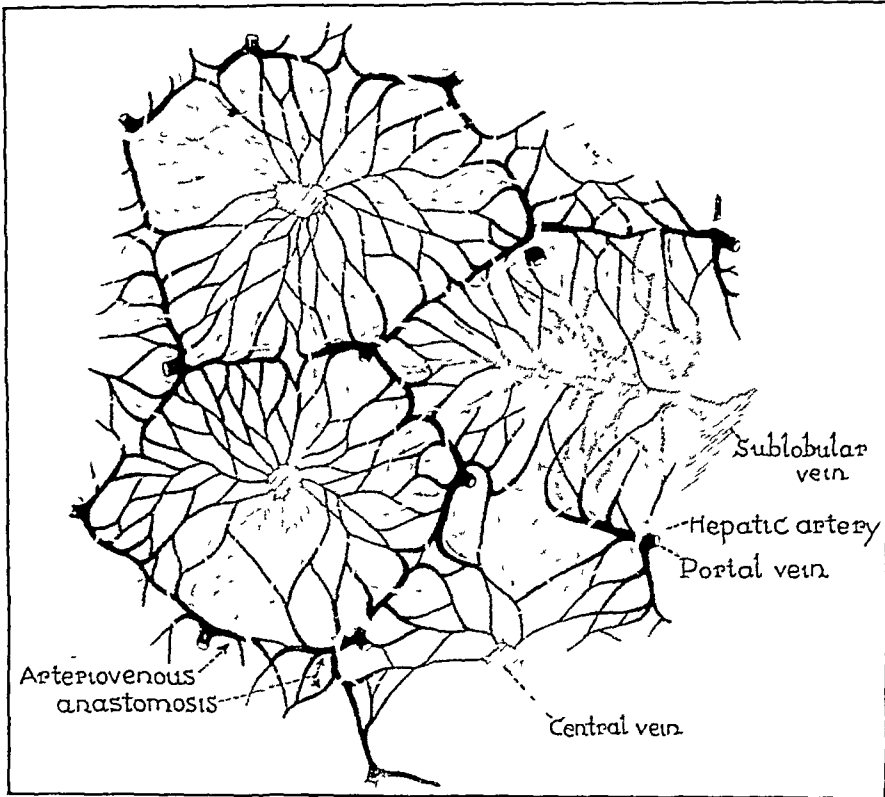


FIG 150 —Circulation in hepatic lobules (From Wakim and Mann, courtesy of Anat Rec)

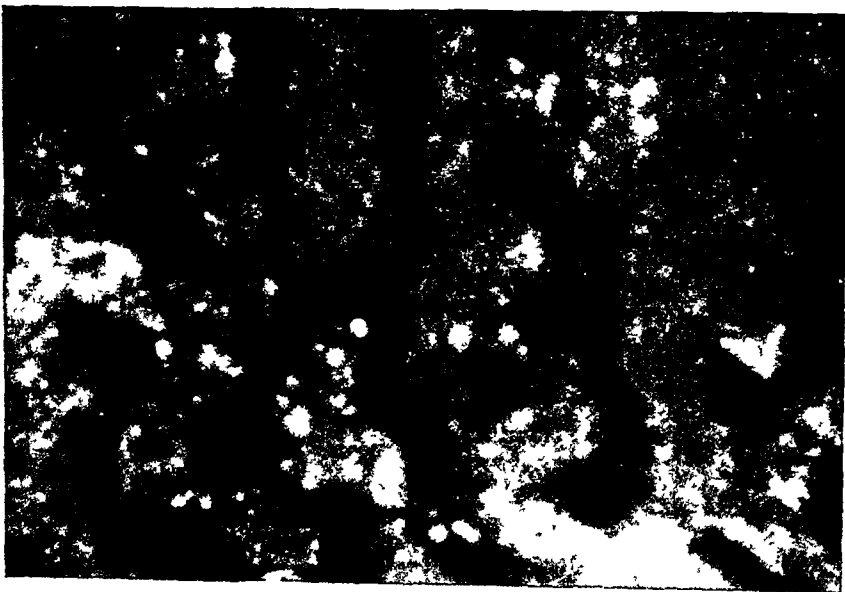


FIG 151 —Photomicrograph showing vitamin A fluorescence in section of normal liver of twenty-nine year old female. Note the cords of liver cells, the fine fluorescent droplets within them especially near their margins, and the heavier fluorescence of 2 Kupffer cells on the right.  $\times 400$  (Popper, courtesy of Arch Path)



and cortical cells are rich in fats and lipoids. The former store vitamin A demonstrable by its fluorescence in ultraviolet light (Fig. 151) and the latter vitamin C (p. 125). Their general arrangement is similar but the columns of cells are straighter and longer in the fasciculi of the adrenal than they are in the liver lobule. Association between cells and blood stream is very intimate. The capillaries become sinusoidal in the inner zone of the adrenal where their endothelial cells are so phagocytic that they are listed as special endothelial cells of the R.F. system. In the hepatic sinusoids the endothelial cells are even more phagocytic. Perhaps degree of

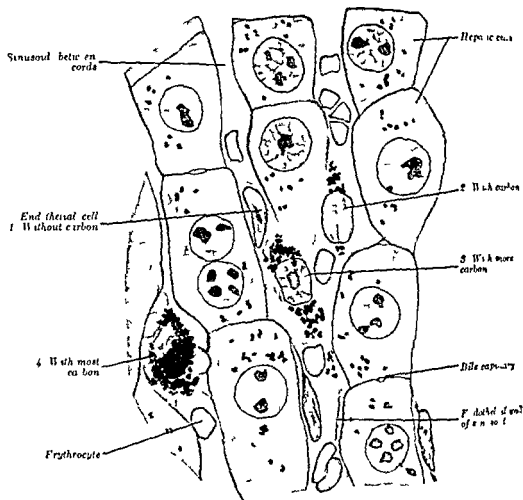


FIG. 152—Phagocytosis of India ink by Kupfer cells in liver of a rabbit. (Holtzman and modified from Maximon in Condry's Special Cytology, courtesy of Paul H. Weber, Inc.)

here a function of opportunity. The rate of flow in the liver may be slower and the incidence of particulate material higher. The engorged cells are unnecessarily named *Kupfer cells*. Stages in the taking up of carbon are presented in figure 152. The fact that they also ingest many substances naturally occurring in the blood helps to make the liver one of the greatest blood filters. There is not much space between the endothelial walls of the sinusoids and the hepatic cells, but such as it is contains tissue fluid, reticulum (Fig. 153) and a few reticular cells. In comparison the tissue fluid approximates very closely to blood plasma for analyses. For that the protein content of hepatic lymph and blood serum is practically identical.

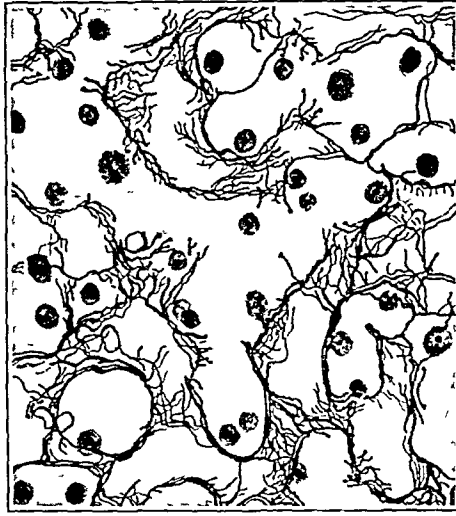


FIG 153 —Reticulum of the liver. (Redrawn from Braus, *Anatomie des Menschen*, Julius Springer )

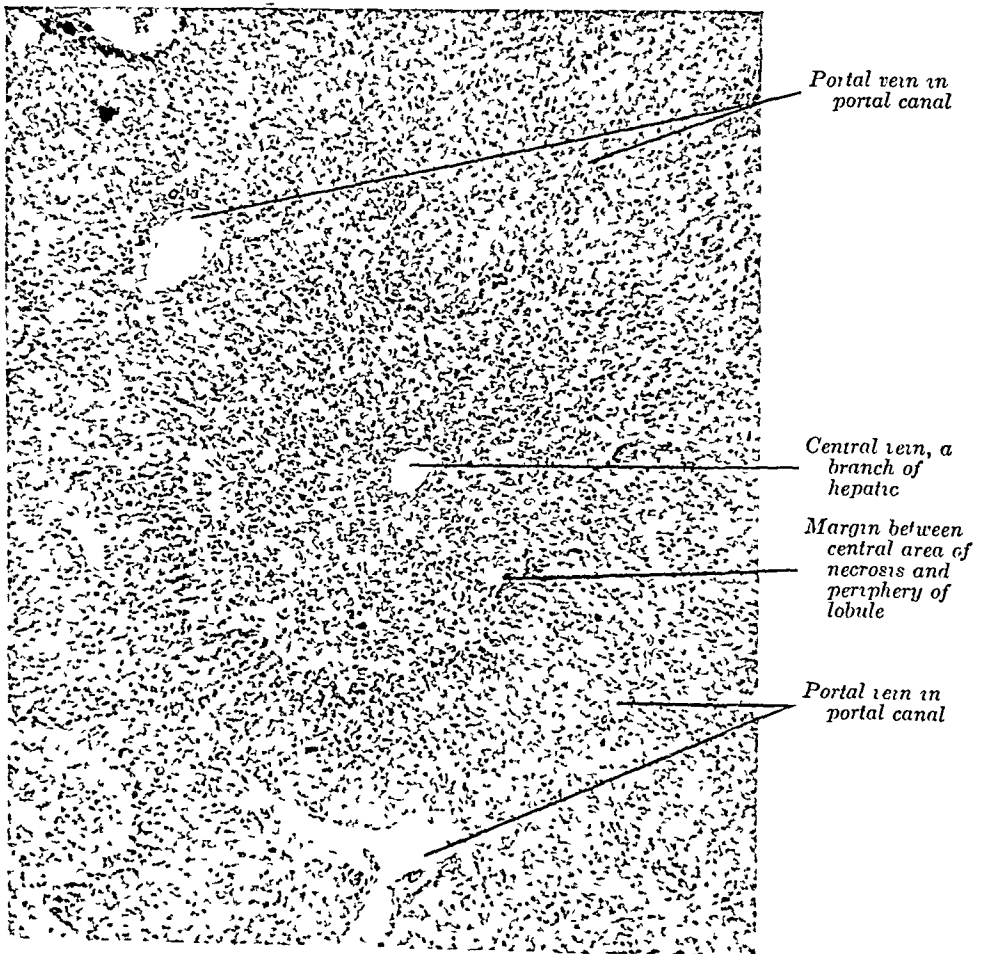


FIG 154 —Central necrosis of liver lobule  $\times 65$  (Dept of Pathology, Washington University, No 3 lent by Dr R E Stowell)

(McCarrell *et al.*, 1941) Tissue fluid apparently seeps in a peripheral direction in liver lobules and is drained out by lymphatic capillaries in the portal canals while in the adrenal cortex it passes centrally for removal by lymphatics of the medulla. The substance of the liver lobules like that of the adrenal cortex is itself alymphatic. The gradient from periphery to center in the adrenal cortex is expressed structurally by the arrangement of cells in fairly clearly defined zones. In the liver lobule the length of the gradient is less and the zones are marked more by a gradually changing behavior on the part of the cells than by outspoken differences in their appearance.

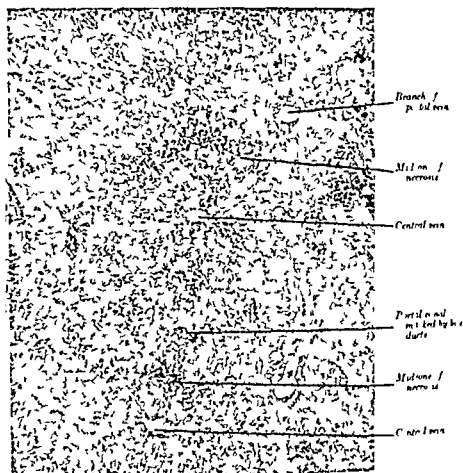


FIG. 153 - Mid zonal necrosis of liver lobule. Yellow fever.  $\times 60$ . (Dept. of Pathology, No. 180 lent by Dr. R. F. Stowell.)

The second task after the location of landmarks is to distinguish these zones within hepatic lobules. They are clearly outlined in some pathological conditions involving necrosis (*Guleros*) the death of cells *en masse*.

Central necrosis is a common lesion in which the cells about the central vein of the lobules are affected. These are the cells least favored with arterial blood and most subjected to passive congestion resulting from failure of blood freely to leave the liver via the hepatic vein. Figure 154 shows a sharp demarcation between the central zone and the remainder of the lobule.

Intermediate (or mid zonal) necrosis is seldom encountered but does occur in

yellow fever for some obscure reason. Close study of figure 155 brings to light the fact that, between little changed cells near the central vein on one side and a portal canal on the other, there is a band of necrosis which is stained more deeply than the rest of the tissue. Examined at higher magnification this is seen to contain degenerating liver cells and infiltrating and dying leucocytes.

Peripheral necrosis is frequently met with in eclampsia complicating pregnancy. Associated with the necrosis, peripheral hemorrhage is not unusual as in the liver represented in figure 156. The peripheral parts of the lobules near the portal canals are outlined by the strong staining with eosin of the closely packed red blood cells.

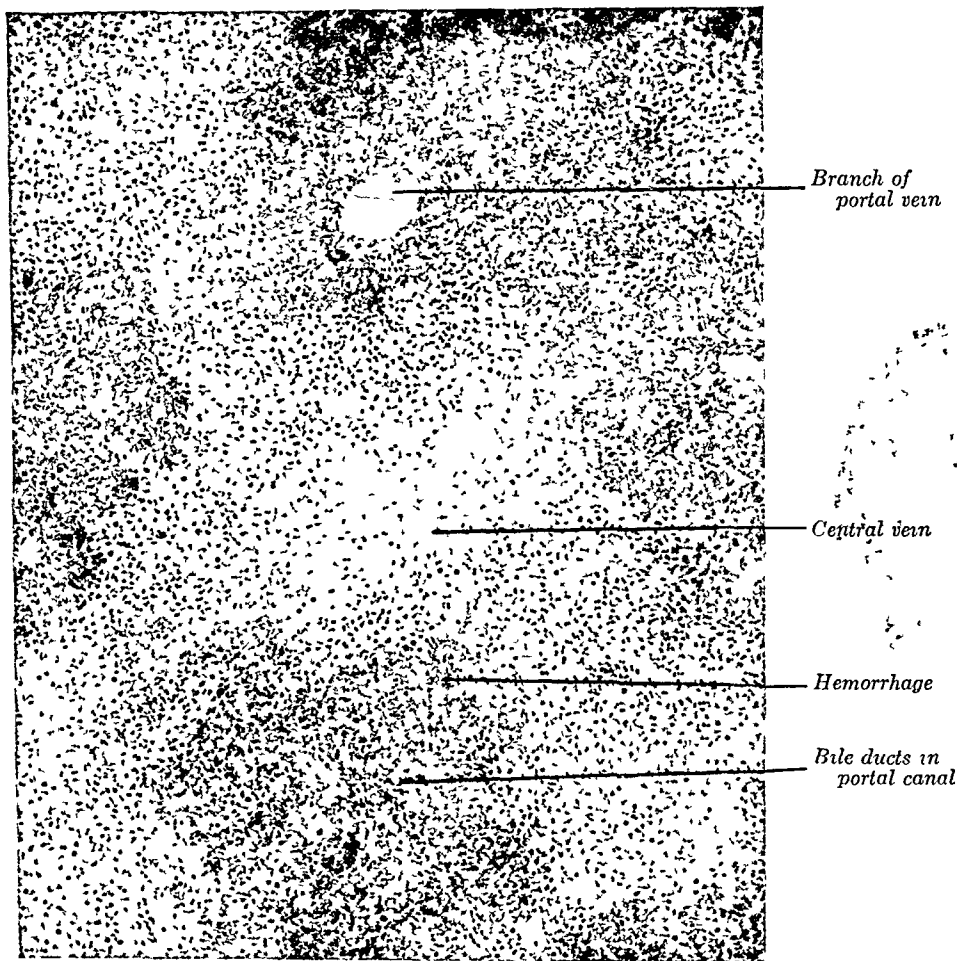


FIG 156 —Peripheral hemorrhage and necrosis of liver lobule in a woman, aged twenty-eight years, who died of eclampsia.  $\times 65$  (St. Louis Maternity Hospital, 12364 lent by Dr. John E. Hobbs.)

There are other data on functional differences in the zones. The mitochondria in the central zone are generally thin and filamentous. Passing toward the periphery they become thicker and shorter. Using them as indicators of activity and working on mice, Noel (1922) has discovered that next the central vein their appearance is little, if at all, modified by radical changes in diet. He calls this the zone of "permanent repose" (Fig. 157). External to it is an intermediate zone of transition capped by a peripheral zone in which the alterations are very striking and which he designates the zone of "permanent function." In a recent paper

Deane (1912) reports no definite relation between mitochondria and fat accumulated in the livers of mice in consequence of a high sugar diet. While on the subject of fat it is significant that this first accumulates in large droplets in the peripheral cells where the rounded spaces originally occupied by it can be detected in figure 155 is examined with a hand lens. Why this is so is not entirely clear. The fact that these cells help themselves before the central ones can do so from the revolving table of the blood stream may or may not be of significance in this particular connection. If the intake is equal one would expect cells with the lowest oxidative metabolism to burn fat least and thus to store it most.



FIG. 157.—Changes in the appearance of hepatic cells of white mice fed 4 fat B & C protein passing from the central vein below up toward the periphery of the lobule. The mitochondria are represented in black. Note their constancy near the central vein and the distinctive changes remote from it. (Redrawn and modified from Noel Thomsen de l'université de Paris. Macon et Cie.)

The storage of carbohydrate in the form of glycogen is a very important function of liver cell. Credit is due to Benley and Gersh (1933) for demonstration of the diffuse condition in which this exists in the cells. When glycogen is first deposited in the cell it is about the central vein. The lobule fills up until the most peripheral cells are included. When glycogen is given up it leaves the peripheral cells first and the central ones last.

The peripheral cells are, as already stated, those most serviceable in regeneration. They are more likely to be binucleated than the cells more deeply situated. Variations in nuclear size are perhaps normally greater in the liver than in any other organ. This is related by Beams and King (1942) to differences in chromosome numbers. Nucleolar hypertrophy and enormous distension of nuclei by fluid, which, latter, limits the chromatin to a thin layer just beneath the nuclear membrane, are phenomena not infrequently seen in the periphery of the lobules, the functional significance of which remains obscure (see figure of Cowdry and Kitchen, 1930). Intranuclear crystals are conspicuous in a few species including dogs (Weatherford and Trimble, 1940).

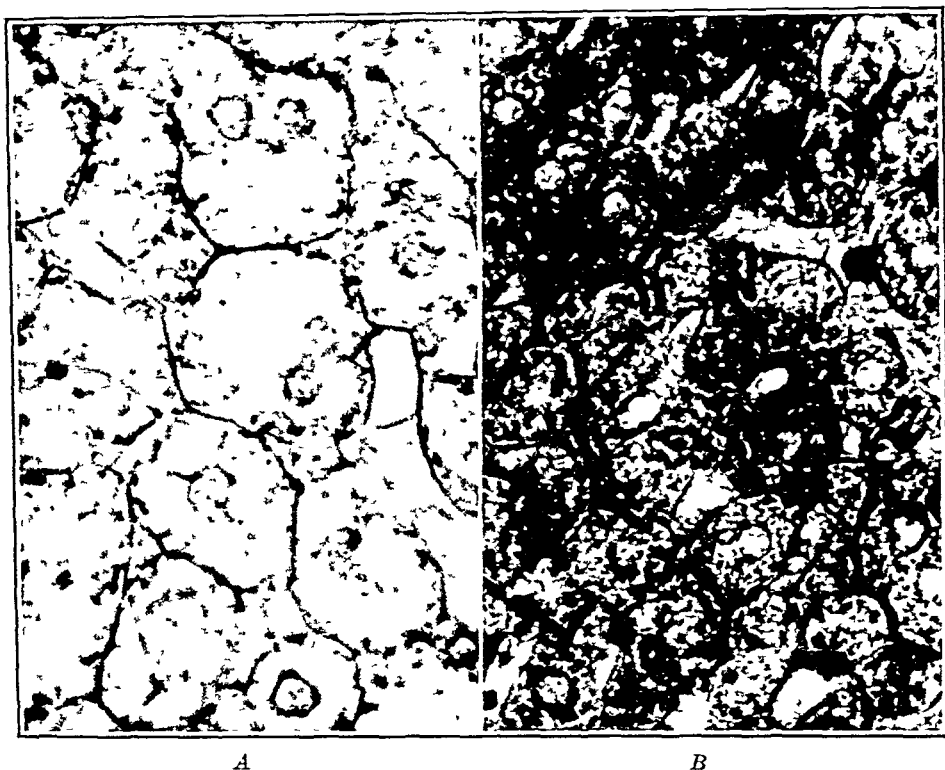


FIG 158 —Rabbits' livers fixed in barium chloride and formalin, soaked in alcohol, treated with benzol embedded, sectioned and stained with acid fuchsin as recommended by Forsgren (1929) A, At height of assimilatory stage (weight of liver, 133 gm, and glycogen content, 12.9 per cent) Bile capillaries narrow, almost empty, liver cells are large, about  $36\ \mu$ , and contain a few bile components B At height of secretion (weight of liver, 50 gm. and glycogen content 1 per cent) Bile capillaries are dilated and filled with bile components precipitated by the barium chloride The cells are loaded with bile components but are smaller in size, having a diameter of about  $16\ \mu$  (Forsgren, J Morph.)

Within the cytoplasm of liver cells one can usually distinguish a somewhat variable basophilic granulation. Its nature remains to be discovered, but a report by Korenchevsky (1941), that the granules are decreased in number and size in rats by gonadectomy and are restored to normal after injections of sex hormones is at least, interesting.

Bile components have been investigated by Fosgren (1929, 1931) and other Swedish workers. At the height of the assimilatory phase in his experiments the liver has doubled its weight, owing, he thinks to increased water content accompanying the intake of substances absorbed from the intestine, and its structure

is represented in figure 155 *A* while at the height of secretion it has lost weight and presents the appearance illustrated in figure 155 *B*. The contrast between the two is noteworthy. In the first glycogen is increased and bile components decreased and in the second the reverse happens. The reciprocal changes are summarized in diagram form in figure 159. He states that "The cells in the periphery of the lobule in which the production of bile begins first and ceases last are more secretory in nature while those in the interior in which the production of glycogen begins first and ceases last are more assimilatory in character."

Because so many substances freely enter the liver cells from the blood and are given by them back to the blood, it is surprising that the bile which they secrete never finds its way either into the blood vessels or lymphatics. Instead it is collected by a special system of bile capillaries. Close examination of a routine prepara-

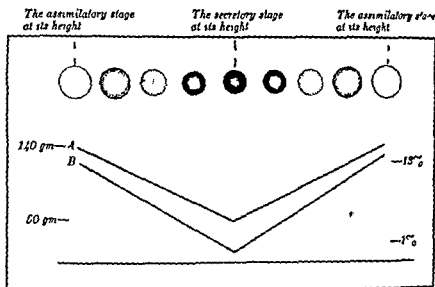


FIG. 159.—Diagram of hepatic functional stages based on experiments with rabbits. The circles represent the lobules of the liver with vena centrals in the center. The white area represents cells that are rich in glycogen but deficient in bile components, the black areas those that are rich in bile components but deficient in glycogen, the stippled areas those containing both bile components and glycogen. A is the weight curve, and B the glycogen curve during the functional stage. (Redrawn and slightly modified from Forster, J. Morph.)

tion often reveal a few of these channels. They are of very uniform diameter and are always between liver cells and remote from sinusoids. At the midpoint in the often straight line of apposition of two liver cells a bile capillary may occasionally be distinguished by its convex lens shape in cross section. It has the appearance of being produced by furrow-like depressions of the cell membranes of sharp or V-line and equal depth in the two cells. When appropriate methods are employed bile capillaries can be observed to empty into the bile ducts in the portal canals (Fig. 164).

The interlobular ducts in turn run together eventually to form the common hepatic duct which receives the cystic duct from the gall bladder and continues as the common bile duct emptying into the duodenum.

The discharge of bile is partly controlled by the gall bladder in those animals

which possess one. The human gall bladder is a pear-shaped sac placed as a sort of shunt at the side of the biliary tract. In rare cases it is congenitally absent. For explanation see Boyden (1929). The bile backs up into it for storage between meals, which brings about a striking change in the pattern of the bladder mucosa (Boyden, 1925).

Graham's (1926) account of the discovery of a means of visualizing the gall bladder *in vivo* by intravenous injection of tetraiodophenolphthalein and roentgen-ray study will prove interesting. Structurally we can recognize in the wall of the gall bladder (1) A tunica mucosa which is much folded. It is made up of a single layer of columnar epithelial cells which ordinarily do not possess a striated distal border like those of the intestine but are nevertheless absorptive. (2) A tunica muscularis in which the fibers are not arranged in distinctive layers, as in the intestine, but in the net-like formation characteristic of the urinary bladder. This is pervaded by numerous elastic and collagenic fibers with a few fibroblasts. (3) A tunica serosa made up of peritoneum on the side which is exposed and is not embedded in the substance of the liver.

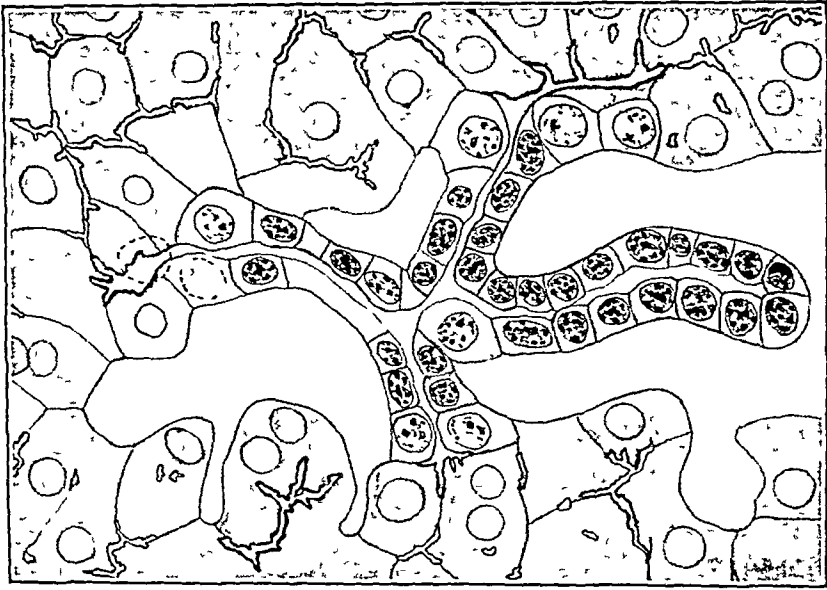


FIG 160 —Section of liver of a man, aged forty-three years, fixed in Muller formol and stained with iron hematoxylin. The bile capillaries between the liver cells connecting with the bile ducts in a portal area are heavily outlined in black.  $\times 1050$  (Redrawn from Clara, *Ztschr f mikr-anat Forsch*.)

When fat enters the duodenum, bile is expelled from the bladder even when all the nerves are cut and further, this is caused by the hormone, cholecystokinin. There is, besides, a rich nerve supply from the vagus and splanchnics. It is difficult to determine the special parts played by nervous and hormonal regulation. The gall bladder capacity is only about 50 cc and approximately 1000 cc of bile are produced every twenty-four hours. It stores and also concentrates the bile by dehydration. The concentration in the dog may be eight or ten times. One recalls the condenser action of the colon. The lymphatic drainage of the gall bladder is a matter of considerable practical importance. It connects with that of the liver (McCarrell *et al.*, 1941).



## SUMMARY

The pancreas is at once an exocrine gland pouring enzymes into the duodenum and an endocrine discharging a potent hormone (insulin) back into the blood. The exocrine portion consists of the acini and their ducts. The structure of the acinar cells and the changes they exhibit during secretion have been discussed in detail because they are so favorable for observation and experiment. Many of the data mentioned apply to other gland cells. The entire cell must be regarded as forming the secretion not simply the mitochondria Golgi apparatus neutral red staining granules or other single microscopically visible components. The act of secretion may be initiated by nerve stimulation various drugs and physiologically by the hormone secretin which reaches the gland by the blood stream from the duodenal epithelium. The endocrine part is made up of scattered clots of cells which contain granules that stain intensely with neutral red by which property they can be clearly distinguished from the acinous tissue. At least three types of islet cells are recognizable A B and C. The B cells seem to be most directly concerned in the manufacture of the insulin which facilitates the metabolism of sugar. When the pancreas is removed this hormone is not produced sugar accumulates in the blood and diabetes sets in.

The liver is the jack-of-all trades of the body established in a strategic position between the great area of intestinal absorption—supplemented by the largest mass of reticulo-endothelial tissue in the body the spleen—and the heart which pumps blood to the entire organism. It is a digestive gland for it secretes bile salts into the gut and kidney for it excretes bile pigments cholesterol and other waste products into the gut for removal in the feces a blood filter, for it removes particulate matter from the circulation and a storehouse, for it accumulates glycogen, protein complex vitamin A and fat. No one would venture a complete list. This singular organ also serves as an assistant to the kidney by making urea to the mechanism of blood coagulation by forming fibrinogen and so on. It was a blood former in the embryo but relinquished this activity to other organs retaining however the ability to develop blood cells in exceptional circumstances. Regulation is brought about not only by the nervous system and the incoming arterial blood but also by the portal stream of venous blood—an additional factor not operative in other organs. Inseparable from these varied activities is the necessity for adjustment to many conditions some of which may be injurious. The liver is possessed of an effective physiological reserve. Large portions can be destroyed or removed without interfering with the performance of its duties. Replacement of incapacitated cells by multiplication of the survivors takes place very rapidly.

## CHAPTER XII

### RESPIRATORY SYSTEM

For the maintenance of the body, supplies are essential and waste must be eliminated. The digestive system provides food and drink and the respiratory system oxygen and both give off some waste materials. The principal areas of absorption are the villi of the small intestine and the alveoli of the lungs. Both are lined by a single layer of highly permeable cells, in close association with the blood stream, and likely to take in harmful substances as well as useful ones if exposed to them. Both are situated at a considerable distance from the entrances. Some of the protective mechanisms interposed between the villi and the outside world have been described. Those operating between the alveoli and the external environment are equally fascinating and important. The hazard is, of course, not limited to the villi and the alveoli, for the tracts leading to them are even more immediately exposed to injury and to invasion by pathogenic organisms. Supplies the body must have, but at a price, in the form of the risk run, which is not to be ignored.

**Nasal Passages** —The mucous membrane which lines the nasal passages and covers the turbinates must withstand the impact of respired air laden, as it often is, with bacteria, dust, fumes and gases. This air may be dry, or moist, at a subzero temperature, or very hot. Discharges from the eye entering by the lachrymal ducts trickle down over the membrane, especially when the eye “waters” owing to some form of irritation. No other living cells are so directly exposed. As we all know, infections are frequent. The penetration of this barrier might well have been prevented had the passages been lined with a relatively impermeable stratified squamous epithelium like that covering the external surface of the body, but, the prime desideratum is the protection of the pulmonary absorptive surface, which could not be so effectively done if dry, dead cells lined the passages instead of living, reactive ones. Moreover, the sense of smell was acquired when our forefathers were aquatic. For its perception the surface must be wet. As it is, the mucous membrane is made up of layers of cells of variable nature and thickness, the number depending upon environmental conditions (Figs 161 and 162). In front of the turbinates, where the exposure is great, it may look something like the skin (Fig 161, *a*). It is only further back and in more vital parts that goblet and ciliated cells are found. In the sheltered interior of the sinuses the epithelium is thinnest (Fig 162, *c*). That the epithelium is highly adaptable to alterations in ventilation consequent upon surgical interference has been shown by Hilding (1932). He observed a change to the stratified squamous type with overventilation and an increase in goblet cells after plugging of the base.

Here, as elsewhere, the epithelium is backed by a *tunica propria* which gives space for blood supply, nerve supply and lymphatic drainage. In it also are tucked away small branched tubulo-alveolar glands, which are usually classified as serous, mucous, or mixed. These undoubtedly produce a watery fluid, and mucus, but difficulty is often experienced in deciding as to the exact nature of any particular gland, because even in those in which mucigens can be found, the nuclei of the secreting cells are not compressed against the proximal cell margins in the usual manner. It is quite possible that individual cells yield both serous and mucous

products. The tunica propria also binds the epithelium to the bony and cartilaginous retaining walls. Since these are immovable, muscle fibers are absent and elastic tissue is limited. Only passive swelling occurs resulting either from accumulation

## DIVISIONS OF RESPIRATORY TREE

	<i>Tunica mucosa</i>	<i>Tunica propria</i>	<i>Supporting structures</i>
Nasal passages	Thick stratified squamous at entrance pseudostratified elsewhere with many ciliated and goblet cells except for absence of typical respiratory ciliated cells in olfactory area	Connective tissue binding epithelium to rigid walls, elastic fibers and muscle not needed. Mucous serous and mixed glands	Cartilage anteriorly the other walls bony
Nasal sinuses	Single layered or pseudostratified epithelium made up of ciliated and goblet cells	Less connective tissue binding epithelium more closely to walls. Elastic fibers and muscle not needed. Mucous serous and mixed glands present but less developed	Bony walls
Pharynx	Ciliated and supplied with goblet cells above stratified squamous like mouth and esophagus below	Connective tissue more elastic, constrictor muscles well developed. Mucous glands only. Tonsil formation	Vertebral column behind, palate and epiglottis in front
Larynx	First stratified squamous, then ciliated stratified squamous and ciliated again	Much reduced epithelium very close to underlying cartilages and special elastic bands. Few glands. Musculature highly differentiated. Internal and inferior laryngeal nerves	Special cartilages and framework for necessary constriction of lumen
Trachea	Simple columnar pseudostratified or stratified ciliated goblet intermediate and basal cells	Wider. Mucous serous and mixed glands. Much elastic and collagenic tissue a little muscle especially between tips of cartilages	Incomplete circular cartilages which invest anterior and lateral aspects
Bronchi 1 mm + *	Same but ciliated cells become relatively more numerous	Reduced. Mucous glands disappearing muscle and elastic tissue	Incomplete circular cartilages arranged to completely invest face
Bronchioles 1 mm - *	Ciliated cells dominate goblet cells lost	Further reduced. No glands. Muscle collagenic and elastic tissue proportionally increased	None
Respiratory bronchioles 0.5 mm - *	Ciliated cells partly replaced by cuboidal. On sides appear air sacs lined with respiratory squamous epithelium	Muscle collagenic and elastic tissue	None
Alveolar ducts	Squamous with occasional traces of cuboidal epithelium	Muscle much reduced collagenic and elastic tissue	None
Alveoli (air sacs)	Squamous	Elastic and reticular tissue	None

\* These measurements relate to the collapsed fixed condition of most preparations and are subject to great variation.

of tissue fluid or from vascular engorgement. The turbinates are very highly vascularized. The vessels over the inferior and middle ones and the adjacent parts of the septum form corpora cavernosa, comparable though not identical with those

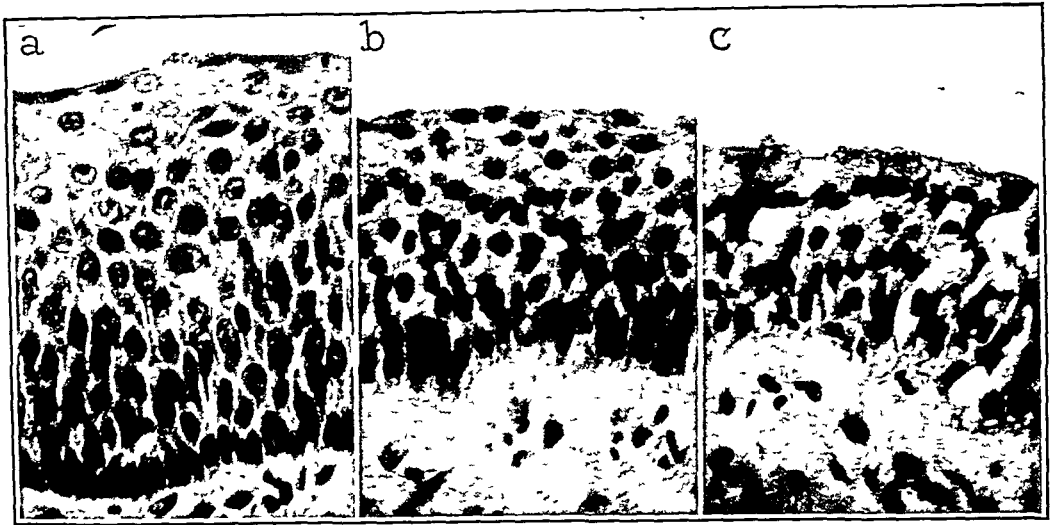


FIG 161 —Regional differences in normal nasal epithelium *a*, Preturbinal region close to the anterior end of the middle meatus, *b*, anterior third of the inferior turbinate, *c*, transitional epithelium somewhat farther posteriorly (Hilding, Arch Otolaryngol)

of the penis. On engorgement with blood the corpora swell up and restrict the passage of air through the nose. It is likely that this is in some cases helpful. The conditions that govern the erectility of these parts are not well known. The possi-

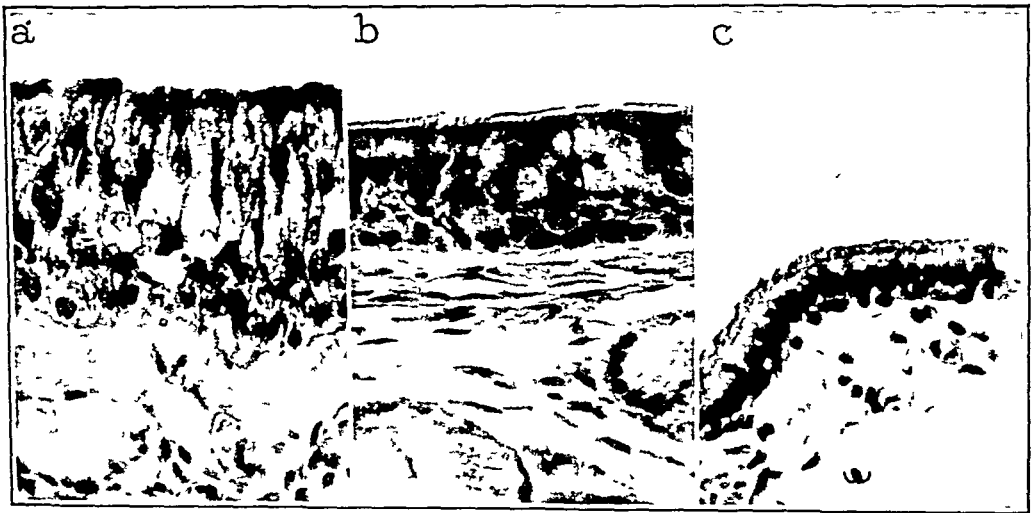


FIG. 162 —Regional differences in nasal epithelium continued *a*, High columnar epithelium from the middle third of the inferior turbinate. This is the type of epithelium found over most of the surfaces of the common meatus, *b*, epithelium from the middle meatus, *c*, entire thickness of the mucous membrane from the frontal sinus of a dog (Hilding, Arch Otolaryngol)

bility of some relation to sexual activities is discussed in an interesting way by Schaeffer (1920). One of the most significant reactions of the tunica propria, both here and in the paranasal sinuses, is infiltration with eosinophiles (p. 28), which

may or may not be accompanied by a general increase of eosinophiles in the blood stream.

Though the nasal mucous membrane does not exhibit absorptive cells with cuticular borders similar to those in the small intestine, the intake of some substances is very rapid. A graphic demonstration of the absorption and lymphatic drainage of trypan blue can readily be made by a method described by Yoffee (1941).

Clearance of the nasal passages is similar to that of the entire airway up to the ends of the bronchi. A protective investment is formed on the surface of the epithelium by the secretion of the goblet cells and the mucous glands of the tunica propria. It is probable that the goblet cells are discharged by contact stimulation or through a local reflex, while the glands are under direct nervous control for both are apparently regulated in this way in the trachea.

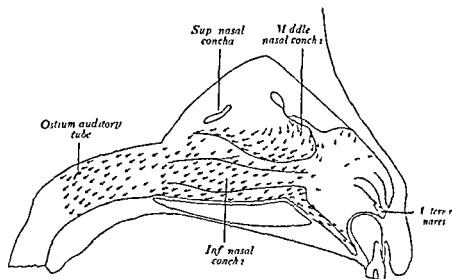


FIG. 163.—Left lateral nasal wall of a monkey, *Macacus rhesus*. Arrows indicate the direction of clearance of mucous layer by ciliary action. (Redrawn and modified from Lucas, *Am. J. Anat.*)

The secretions do not heap up, but are moved on toward the pharynx by the beating of the cilia and occasionally by purposeful forced expiration. The direction of the streams has been investigated in the monkey by Lucas (1932a). They converge and pass near the opening of the Eustachian tube, infection of which might be more frequent were it not that entry is discouraged by another group of cilia in it beating downward. Figure 163 illustrates clearly the distribution and direction of movement of cilia on the lateral wall of the nasal cavity. The cilia is ciliated up to approximately the same level. The ciliated cells are covered with a layer of serous fluid which offers very little resistance to their vibration. Only the ends of the cilia are in touch with a film of mucus which they shift in the directions indicated by the arrows. Without the lubrication afforded by serous fluid the viscosity of the mucus would prevent their movement. Bacteria particles, dust and other foreign material adhere to the mucous sheet and are removed. The nasal vestibule is not ciliated but its epithelium is unusually thick. The olfactory area above is likewise devoid of vibratile cilia. Here the epithelium can not be

squamous because that would preclude stimulation of the sensory olfactory cells resident in it. Inhaled chemical substances must come in intimate contact with it and something may be gained by letting them come to rest. Clearance is, neverthe-

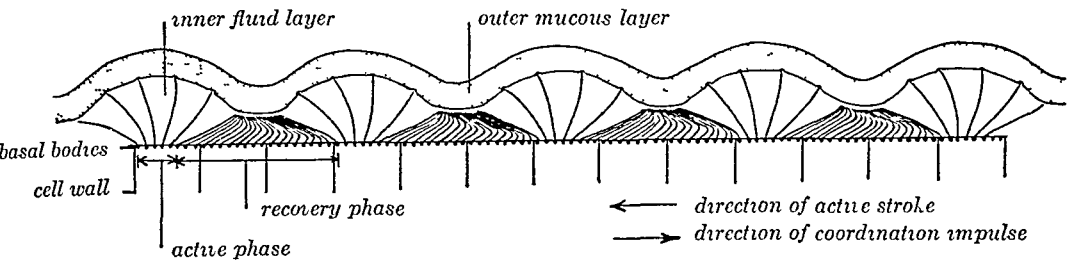


FIG 164 —Diagram of ciliary action (From Lucas, A. M. and Douglas, L. C., courtesy of Arch Otolaryng)

less, accomplished slowly by the action of cilia in the nearby epithelium, shifting the watery fluid containing a little mucus that covers the area devoid of cilia. There is reason to believe that this area of minor resistance is the site of entry of the virus of poliomyelitis and perhaps of others also (Fig 165)

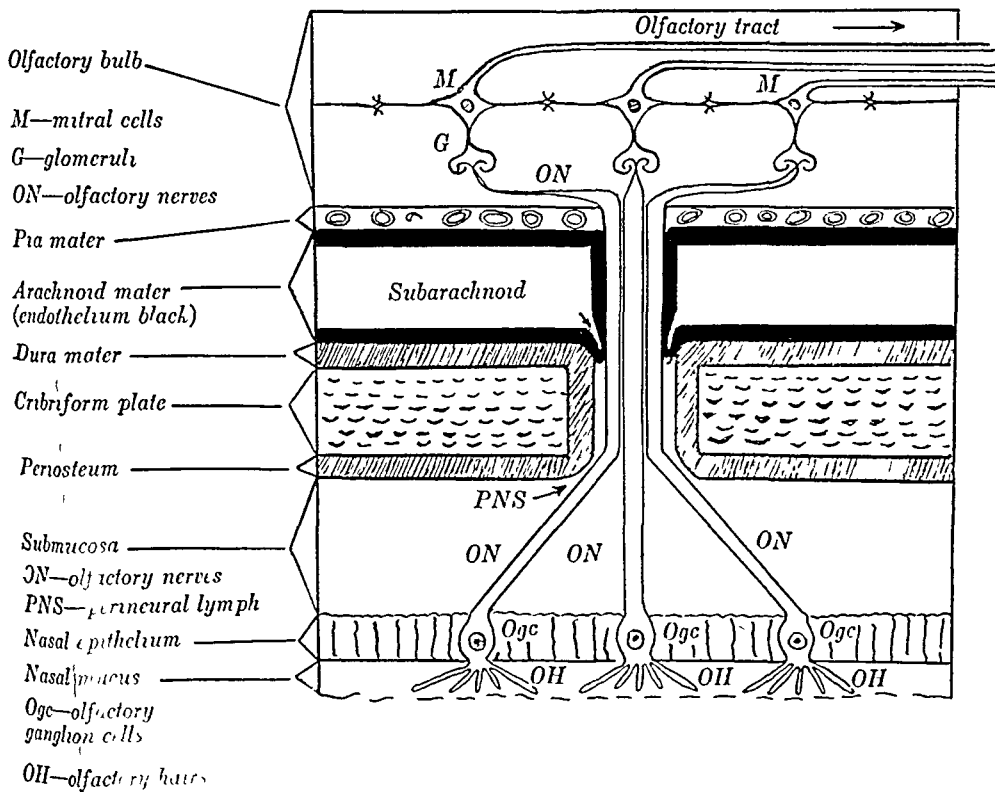


FIG 165 —Diagram of the olfactory mucous membrane, olfactory nerves and connections to show the lines of penetration of an invading virus like that of poliomyelitis (1) through the substance of the nerve cells and fibers or (2) in the tissue spaces from the submucosa along the nerve fiber (PNS→) entering the closed subarachnoid space at the point (↘) (Redrawn from Labor, Medicine)

Small injuries to the nasal mucosa are quickly repaired. Epithelial cells bordering the injury differentiate and divide by mitosis, then migrate over the denuded area, or over a blood clot, if one is present, as a thin squamous sheet (Fig 166)

This squamous layer becomes stratified by further migration of bordering cells and by multiplication of cells already present. Later the cells of this stratified epithelium differentiate into the normal nasal epithelium. The tunica propria is replaced by scar tissue in which there is very little glandular regeneration. The injuries are usually very free from infection except where the underlying cartilage or bone has been exposed (see Boling 1935)

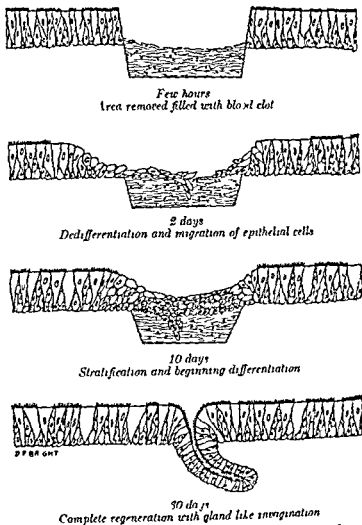


FIG. 166 - Diagram of stages in repair after removal of piece of nasal mucous membrane from a sheep (Courtesy of L. R. Boling)

Evidently the drainage of the nasal passages depends upon several factors. (1) The cilia must vibrate normally. Movement is excited by direct stimuli of many sorts and Lucas (1933) has observed that in the frog it is under nervous control. He also discovered that the cilia are often at rest when examined *in situ* in the same animal and special care is taken to avoid any kind of stimulation. The usual notion that their activity is necessarily continuous may therefore be erroneous. (2) An adequate supply of serous fluid of the right sort is necessary. (3) Lucas has frequently observed that the mucous sheet may fail to move although the cilia are beating vigorously. The stagnation of potentially harmful materials may be due to a change in the mucus. On the other hand when the sheet is moved

normally it is able to bridge small denuded or injured areas of ciliated epithelium because its viscosity is normally so great that it can be pulled over them. If the obstacle is larger, the direction of flow is modified to pass around it, but the adjacent cilia do not change the direction of their beating to facilitate in any way the passage of the mucous layer in the new course. Violent clearance of the nose is brought about by a sneezing reflex through the sensory terminals of the fifth nerve in the mucosa. See book by Proetz (1941) on "Applied physiology of the nose." He has an excellent motion picture film showing ciliary activity (Dr. A. W. Proetz, Beaumont Medical Bldg., St. Louis).

**Paranasal Sinuses.**—These appendages to the nasal passages are known by the names of the bones involved. maxillary, frontal, sphenoidal and ethmoidal. The *maxillary sinuses* are the largest and the only ones to which the term antrum (G. *antron*, a cavity) is applied. Owing to the fact that the sinuses are blind pockets with small openings, very little air enters them during breathing. The epithelium is thin, consisting generally of only a single layer of goblet and ciliated cells. The tunica propria also is less developed than in the nasal passages, but it contains a few glands of the same types. Blood vessels are likewise reduced, because there are fewer cells to nourish and the necessary heating is at a minimum, since blasts of cold air do not traverse them. Ordinarily their contents are bacteriologically sterile. They flush themselves out in the same manner, by ciliary action. The direction of transport in the maxillary sinus has been mapped by Lucas (1932a). It does not proceed straight toward the ostium, but in the form of an ascending spiral. An artificial opening never gives as good drainage as the ostium for the reason that this original direction of ciliary movement toward the ostium is maintained (Proetz, 1932). Those wishing to pursue this subject further will find helpful reviews of progress in the *Archives of Otolaryngology*. One by Salinger (1942) on the paranasal sinuses gives valuable leads.

**Pharynx.**—This is used by both the respiratory and digestive tracts and partakes of the properties of both. It is usually held open in an up and down direction, to facilitate breathing, by attachment of the pharyngeal aponeurosis to the rigid bones at the base of the skull, to the mandible, various ligaments and cartilages of the larynx. It receives the mucous drainage from the posterior nares and Eustachian tubes above and from the larynx below, which is normally disposed of by swallowing.

The surface epithelium of the upper, nasal part, of the pharynx is ciliated and supplied with goblet cells. In the tunica propria are glands which yield the required serous and mucous lubricants. There are also many lymphocytes which may assume the proportions of a pharyngeal tonsil. In the lower oral part, the epithelium is, on the contrary, stratified squamous, closely resembling that of the mouth and esophagus, while the glands of the tunica propria are wholly mucous. On the whole, however, the pharyngeal surface is but poorly supplied with secretions keeping it wet. It is by drying of this surface and of the epithelium in the back part of the mouth that the sensation of thirst is evoked. The same region is a favorite site for beginning infections and a feeling of dryness is often complained of, but seldom of thirst.

Food is forced across the airway from the mouth in front into the opening of the esophagus behind by constrictor muscles. When this happens the opening of the larynx, known as the glottis, is closed by the action of a vagus reflex. It is also closed reflexly through the fifth nerve when certain gases enter the nose, through the ninth nerve when they irritate the pharynx and consciously to maintain pressure



delicate boundary typical of an alveolus the passage can be identified as a respiratory bronchiole.

**Alveolar Ducts**—These lead from the respiratory bronchioles to the alveoli. They are easily recognizable in sections by the contours of their wall. On the inner surface are inward folds the innermost parts of which are thickened by delicate bands of smooth muscle with the result that in sections they look knob-like (Fig. 170). Alveolar ducts are the last divisions of the air way to be supplied thus incompletely with muscle. Alveolar sacs or better simply alveoli bud off from their sides. But in a misguided search for minutiae some people describe chambers or bullae interposed between the alveolar ducts and the alveoli which they call atria (Lancaster courts or hells).

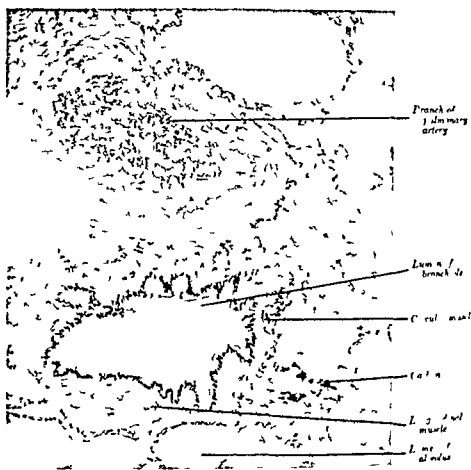


FIG. 169. Bronchiole and pulmonary artery in lung of a sixty-four year old female autopsied three hours after death. Anatomical findings sufficient to explain cause of death. (Zucker formalin fixation, H & E stain  $\times 10$ ). (Dept. of Pathology, No. 1040, lent by Dr. R. F. Stowell).

**Alveoli.** These are the ultimate divisions of the respiratory tree—the leaves so to speak. It is evident that their walls are of exceeding thinness and that the capillaries of the pulmonary artery carrying venous blood for aeration come in intimate association with the alveolar air (Fig. 170). Most of the lungs prepared are from partly collapsed lungs obtained at autopsy or operation so that the alveoli are not normally distended.

It is known that the walls of the alveoli are elastic and that their internal surface is moistened with a film of fluid. Although we are inhabitants of dry land, we breathe through water like our remote ancestors. Examination of thick sections reveals the fact that in some cases the walls are incomplete being perforated by tiny holes, the alveolar pores (Fig 171). These are well described by Macklin (1936a). But the actual composition of the limiting membrane is still in active debate (Ham and Baldwin, 1941). In fetuses the membrane is made up of cuboidal epithelium and it has been supposed, when the baby is spanked and the lungs are first expanded, the cells become greatly stretched but nevertheless continue to function as a thin epithelial sheet. Yet in children and adults it is difficult, sometimes impossible, to

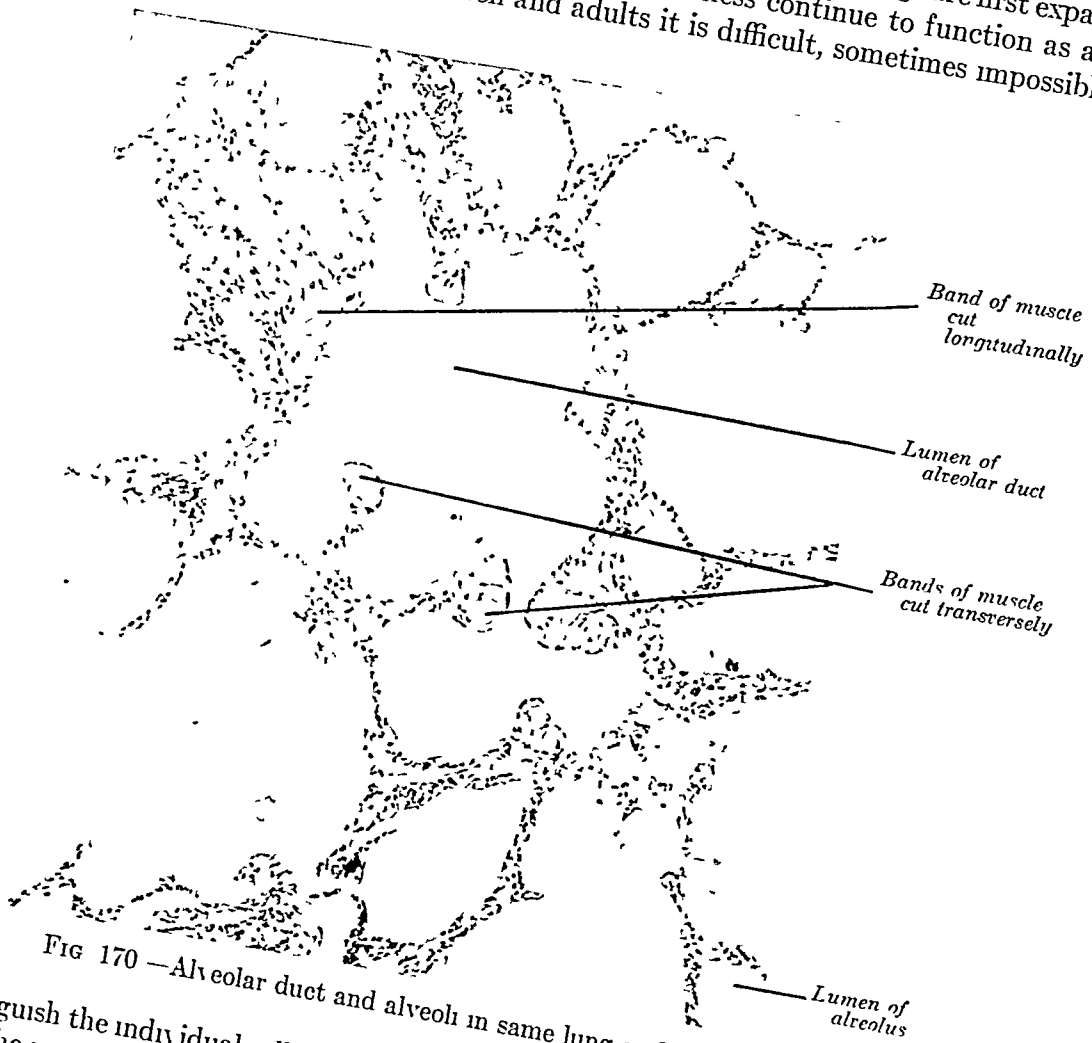


Fig 170 —Alveolar duct and alveoli in same lung as figure 169  $\times 105$ .

distinguish the individual cells credited with making up the membrane. Some claim that the membrane is composed partly of cells and of thin non-nucleated plates but the latter are just as elusive as the cells. Others are quite uncompromising, think no epithelial membrane exists and that we are merely dealing with a condensation of connective tissue and naked capillaries directly bathed by tissue and alveolar fluids (Fried, 1934, Josselyn, 1935). In the absence of a membrane the extravascular tissue fluid and the alveolar fluid must be one and the same and this would be the only known place in the body where tissue fluid is directly exposed to the air. Impressive, however, is the fact that an accumulation of fluid can lift a mem-

brane-like structure off from the underlying tissue as is illustrated in figure 172. It may also be significant that in certain tumor like growths of the lungs of animals (Cowdry 1925 a b) and also of humans the alveoli become lined with cuboidal epithelium. This is represented in figure 173. On the right is such an alveolus with a papillomatous growth entering from the side the epithelial cells of which are even columnar. On the left are other alveoli in the largest of which the enlargement of epithelial cells is irregular some quite tall ones being separated by flatter ones. These epithelial cells must either arise by multiplication and swelling of previously existing greatly flattened cells or by the wholesale migration of epithelial cells from the bronchioles. That epithelial cells can shift in from the sides to cover connective tissues denuded of their usual epithelial protection is well known. The margins of



FIG. 171 — Multiple alveolar pores from the lower border of the lung of a man aged forty. The wall of an alveolus is viewed vertically. The pores are clear openings of variable size with rounded edges.  $\times 370$  (Macklin courtesy of Arch. Path.)

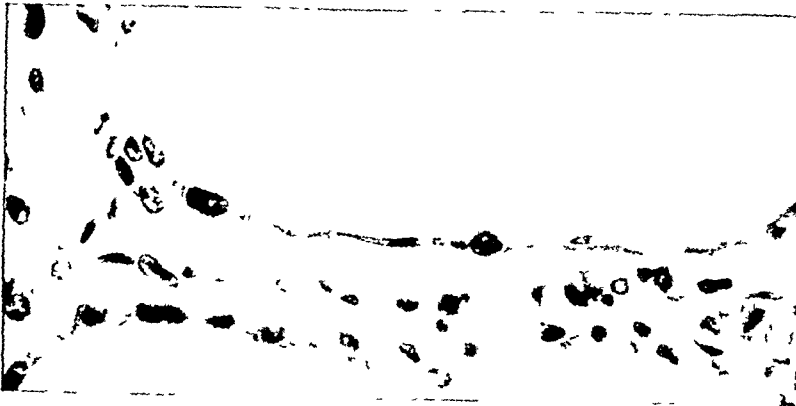
the alveolar pores indicated in figure 171 do look as if they are constituted by a definite membrane (fig. 171) but to assert that it is epithelial would be dogmatic. Because in most cases an epithelial membrane can't be seen is a natural deterrent against blind belief in its existence but the presence of a plasma membrane limits the cytoplasm of all cells is not questioned owing to our inability to distinguish directly with a microscope. Convincing evidence of a different order demonstrates that it is a real structure of surpassing importance. As yet the other evidence is lacking concerning the precise limits of the alveolar wall but for the present it seems likely that the alveoli are surfaced by an epithelial sheet of exceeding delicacy.

Beneath this hypothetical sheet is the capillary bed some elastic fibers and connective tissue cells but no lymphatic capillaries. It will be remembered that lungs

phatic capillaries are also lacking in the substance of liver lobules—another location where exchange between blood and tissue fluid is especially free. This tissue fluid presumably seeps into the alveoli as well as toward the bronchioles where it is picked up by lymphatic capillaries. On occasion, the alveolar fluid is, by exudation, greatly



A



B



C

FIG. 172 —Three sections from a series which show the epithelium slightly raised from the surface of the alveolar wall by the exudate beneath it  $\times 500$  (Miller, in Cowdry's Special Cytology, Paul B Hoeber, Inc.)

increased over the normal (for direct observations see Tarry, 1926, 1936) so that in pneumonia the individual may die from what might be described as drowning the water preventing him from breathing. Cells can also enter the alveoli. Some of these are ordinary neutrophils, others are macrophages. The source of the latter is in question. A few investigators contend that they are not of hematogenous or connective tissue origin, but are derived from the epithelial lining and hence properly may be designated 'epithelial phagocytes'.

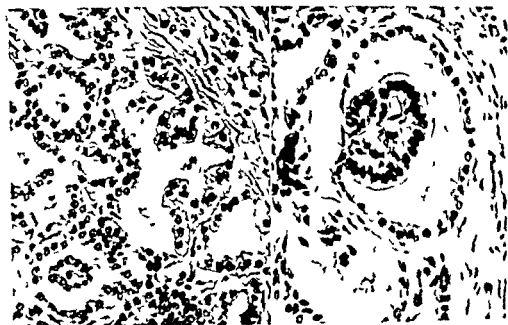


FIG. 173 — Hyperplastic alveolar epithelium in a sheep afflicted with a tumor like disease (jagziekte). Zenker fixation H & E.  $\times 310$

**Blood Supply** — Nothing need be said about the blood supply of the upper respiratory tract but the lungs are like the liver, peculiar in the receipt of a large amount of *venous blood*. Since vessels leading from the heart are termed arteries the vessel carrying this blood from the right ventricle to the lungs is known as the pulmonary artery. Its structure is that of an elastic artery and its branches follow the bronchi and bronchioles and break up into the capillaries in the walls of the alveoli which have been mentioned as well as into others beneath the pleura. Aerated and therefore arterial blood is collected from these capillaries into the pulmonary veins and returned to the left auricle.

*Arterial blood* for the tissue must also be nourished arrives via the much smaller bronchial arteries from the thoracic aorta or intercostal arteries. These supply capillaries to the bronchi, bronchioles and related structures including the walls of branches of the pulmonary artery and the subpleural connective tissue. Venous blood from these capillaries is drained chiefly by the pulmonary veins which consequently conduct a little venous blood in addition to the large volume of arterial blood and to a minor extent by the bronchial veins opening on the right side into the azygos vein and on the left into the accessory hemiazygos or highest intercostal vein.

Identification of these vessels in stained sections may be possible. Branches

of the pulmonary vein do not closely accompany the bronchioles but run at a distance from them through the connective tissue framework of the lung. When two arteries are encountered beside a bronchiole the larger one is probably a branch of the pulmonary and the smaller one a branch of the bronchial, which, latter, is in structure a typical muscular artery. A venule near a bronchiole is probably a tributary of the pulmonary vein, but, nearer the hilus of the lung, associated with large bronchi, such venules may be branches of the bronchial vein. When the orientation of the section of a bronchus is known it is convenient to recall that the pulmonary artery is ordinarily above and behind it, while the pulmonary vein is in front and below. The blood supply of the lung, like that of the liver, is a favorite question on State and National Board examinations.

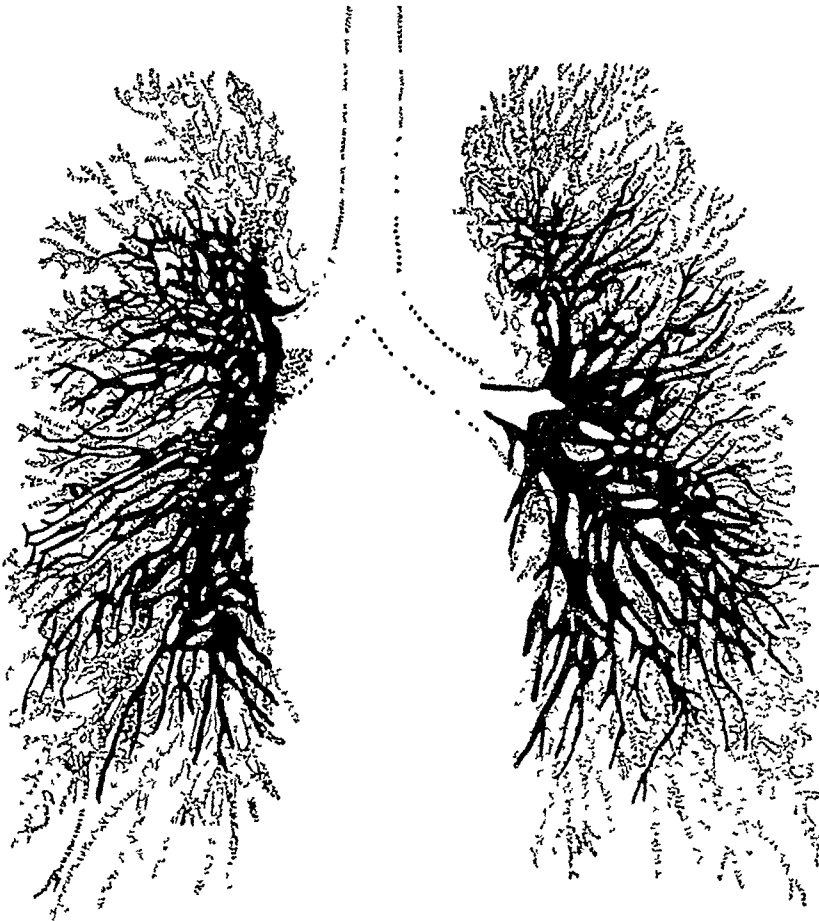


FIG. 174.—In this superposition of a tracing of the bronchial tree of expiration (black) upon that of inspiration (stippled), the roots of the lungs of the two phases have been placed in the same position, which has meant moving the outline of the expiratory phase downward and a little outward. Shows particularly the changes in length of the bronchi in the two phases, and serves also for a study of the interbronchial angles and spaces. (Redrawn from colored figure of Macklin, *Am. J. Anat.*)

**Mechanical Factors.**—On inspiration the thorax increases in size, negative pressure is created, air rushes in backed by atmospheric pressure and tension is increased on the elastic fibers in the alveolar walls and elsewhere. On expiration positive pressure is created, this exceeds atmospheric pressure, air is forced out and elastic tension is reduced. With expansion and relaxation of the lungs there is a

marked change in the length and girth of the bronchial tree (Fig. 174) and their surfaces move against the wall of the thorax. Friction is reduced by the smoothness of the visceral pleura coating the lungs and of the parietal pleura lining the chest and by the thin watery fluid in the pleural cavity between them. When by accident or design an opening is made in the pleural cavity so that the pressure in it equals that in the outside air the lung collapses because the atmospheric pressure which normally opposes the tension on the elastic fibers is balanced. An account by the Macklins (1942) of the ageing of the respiratory system is a mine of useful information.

### SUMMARY

Absorption in the respiratory system is from air and in the digestive system from water. The mechanics are very different. Most of the airway must be held open by bone and cartilage so that when air rushes in under atmospheric pressure owing to expansion of the thorax and the resultant increase in pulmonary space it will not collapse. Much elastic tissue is utilized for the construction of a recoil mechanism which is placed on tension by muscular action in inspiration and aids in expiration. We recall how stretching of the elastic tissue of the aorta on contraction of the left ventricle by recoil helps to maintain the blood pressure while the ventricle is filling. Air reaches the alveoli very quickly, while food is halted ground up and subjected to many digestive enzymes before it is offered to the intestinal cells. The intestinal cells on the contrary are tall columnar structures which are closely wedged together into a firm but pliable membrane. They have the further advantage of being directly protected by a mucous secretion, while the respiratory cells are exposed to the air except for a thin film of water, having in all likelihood about the same silt content as the blood. Most of the airway secretes mucus and cilia are developed for its drainage. The nervous system regulates each respiration to a fraction of a second and provides several reflexes for forceful clearance and for temporarily shutting off all air from the lungs where digestive activities are sluggish and nervous regulation dominant at either end is supplemented by hormonal control particularly in the duodenum and vicinity. Friction is reduced at the surface of the lungs and of the intra-abdominal digestive tract by the development of serous coats moistened with fluid which enables them to glide easily over similar coverings lining the thoracic and abdominal cavities. Both systems are probably lined throughout with epithelium which is capable of regeneration after injuries that are not too serious or widespread. It is through these two gateways that essential materials enter the body and that waste of certain sorts is eliminated. It is also through these portals that special substances may gain entry and cause the living cells so that when confronted again by them the unfortunate individuals respond by a very violent reaction and are said to be allergic or hypersensitive.

## CHAPTER XIII

### URINARY SYSTEM

THIS system performs the central task of stabilizing the composition of the blood. Epithelium again is the star performer. The blood is caused to flow through capillaries beneath a very thin layer of epithelial cells through which filtration takes place. Most of the filtrate is reabsorbed and used over again. The remainder is excreted by the urinary tract. Since the flow is only toward the exterior the entry of harmful substances from without is less of a hazard than in the alimentary and respiratory tracts.

**Renal Lobes.**—The gross appearance of a vertical section through the kidney is illustrated in figure 175. The *lobes* are cone-shaped structures based on the capsule and extending inward. The apex of each cone looks pyramidal in vertical section and fits into a minor calyx of the pelvis whence the urine is drained off. The medulla of each lobe is seen to be striated. Some of the striæ extend out into cortex where they are known as medullary rays. In each lobe are many slender lobules, of which the rays and their continuations in the medulla form the cores.

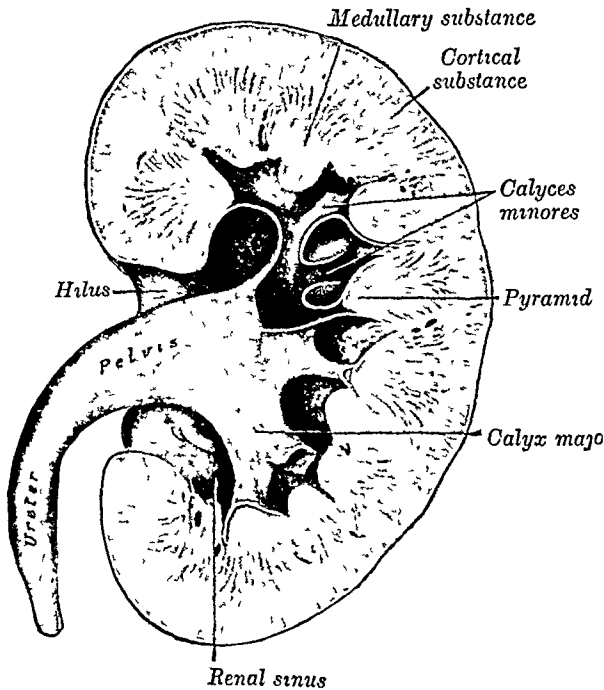


FIG 175—Vertical section through a kidney (Gray's Anatomy)

A pulsating tide of blood enters by the renal artery at the hilus (Fig. 176) and penetrates by interlobar arteries between the pyramids. These arteries fork when they reach the cortico-medullary margin. The resulting branches follow the marginal curve and are therefore called arcuates. From them interlobular arteries spring and pass directly outward into the cortex. In the cortex they give off afferent arterioles to the glomerular capillaries whence the blood is collected by efferent arterioles before being distributed to second system of capillaries which



serves to nourish the renal tissue. Some branches of the efferent arterioles pursue a straight course backward into the pyramids (arteriole recta) before breaking up into capillaries (Fig. 177). The glomeruli hang like 'apples on a tree' (Malpighi). The capillary beds are drained by corresponding venules and veins which leave at the hilus. Individual interlobular arteries supply definite parts of the kidney and do not anastomose with their neighbors. They are typical end arteries, when one is occluded the tissue depending on it suffers acutely. Occasionally these

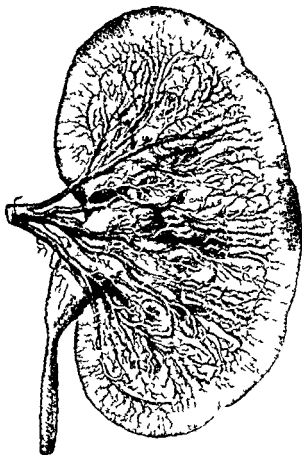


FIG. 176 — Anterior view of left renal artery and the distribution of its branches in relation to the pelvis. Six main branches enter the kidney substance. Only one (the that passes posterior to the pelvis at the hilus). Small arteries from the upper and lower main branches pass behind to the upper and lower calyces. The remainder run anterior to the pelvis and calyces. The small branches to the cortex of the anterior portion of the kidney have not been drawn in order that the large branches and the pelvis might appear more distinctly. (Brodel.)

arteries pass to the capsule and anastomose with branches of the phrenic, adrenal or intercostal arteries or with capsular arteries arising from the renal artery itself in the hilus. (For detailed description of blood supply see Loomis and Jett Jackson 1942.)

**Lobules** — The renal lobules can best be distinguished microscopically when the plane of section is parallel to their length. Figure 175 shows renal corpuscles arranged about a medullary ray constituting the center of a lobule. With them are branches of interlobular arteries and veins.

By careful maceration in strong acid (Microscopic Technique, p 103) the tissue between the renal tubules can be removed and those constituting a lobule, can be slightly spread apart as illustrated in figure 179 Again the periphery of the lobule is marked by the presence of renal corpuscles and the center by their absence and

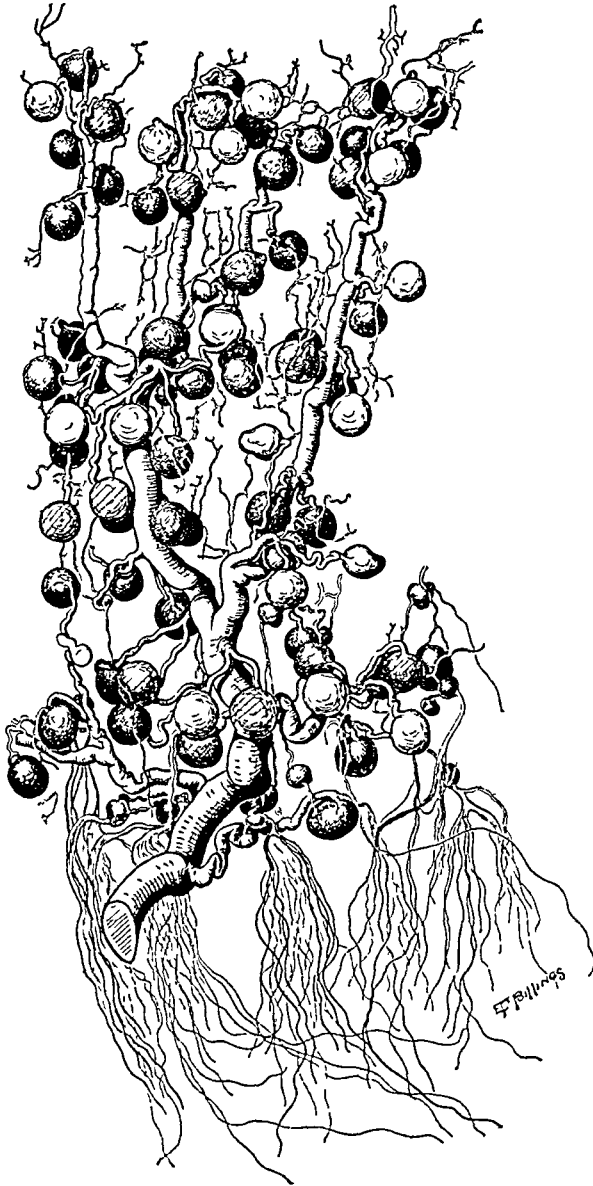


FIG 177 —Celluloid corrosion preparation of the terminal arterial branches of the renal artery of a dog showing arcuate and radiate arterial branches, arteriolae rectae and portions of the cortical capillary net (From Huber, in Cowdry's Special Cytology, Paul B Hoeber, Inc )

the occurrence of many tubules pursuing a more or less straight course toward the pyramid

**Nephrons.**—When a renal corpuscle with its tubule is isolated from its fellows a clearer idea can be gained of its organization (Fig 180). It is a renal structural unit, or *nephron*, and is developed from a different mass of mesoderm (renal blastema) from that forming the collecting tubule (Wolffian duct) with which it connects

and into which it discharges. Each nephron is divisible into a renal corpuscle and 6 segments.

In the renal corpuscle is a mass of capillaries called a glomerulus which occupies most of the lumen. In favorable sections the afferent arteriole leading to it and the efferent one leading away can be distinguished. The former is naturally the larger for it carries whole blood into the glomerular capillaries while the latter carries whole blood minus the glomerular filtrate. The former is also thicker because it is encased in the juxtaglomerular apparatus made up of special cells possessing oval

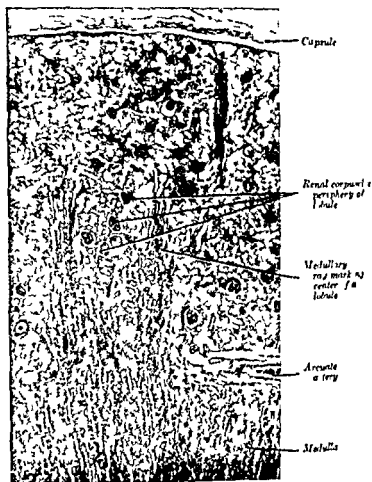


FIG. 178. Kidney of five day old white male who died of colitis. Postmortem one and one-quarter hours later. Formalin Zenker H & E.  $\times 60$ . (Dept. of Pathology Washington University. No. 10312 tissue given by Dr. R. E. Stowell)

vesicular nuclei and conspicuous cytoplasmic granules (Fig. 181). There is another point. The ascending thick segment of the medullary loop comes into close contact with the afferent arteriole and the epithelial cells of its wall next to the artery are distinctly larger than the others. These constitute the macula densa (Fig. 182) which is accused of forming the blood pressure raising substance, renin, as will be mentioned later.

The wall of the renal corpuscle is made up of a single layer of flattened close looking epithelial cells (Bowman's capsule). Continuous with it is another even thinner sheet of epithelial cells which invests the glomerulus and is indeed part of

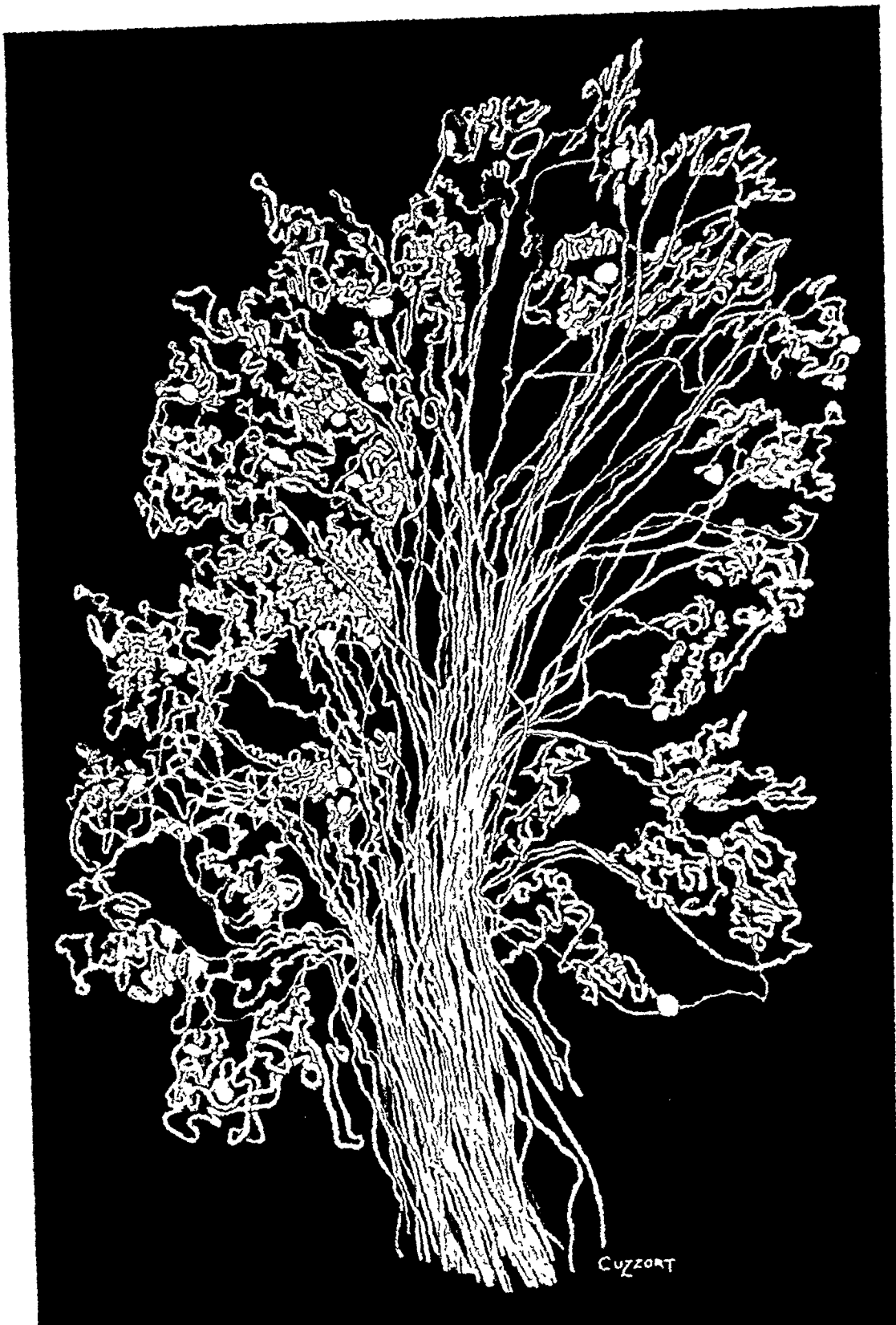


FIG 179 —Arrangement of nephrons in a microdissected lobule of a normal adult kidney about the periphery are the renal corpuscles each surrounded by mass of its own proximal convoluted segment and associated distal convoluted segment. From this cluster two tubules pass to medullary ray, proximal convoluted segment entering the ray and thick segment of medullary loop leaving ray. In the sheaf of straight tubules that compose the ray the junction of collecting tubules can be occasionally seen.  $\times 15$ . (Oliver, Architecture of the Kidney in Chronic Bright's Disease, courtesy of Paul B. Hoeber, Inc.)

the wall pushed into the lumen ahead of the invading capillaries during development. The epithelium of Bowman's capsule is parietal while that covering the glomerular tuft of capillaries is visceral in the sense that parietal peritoneum lines

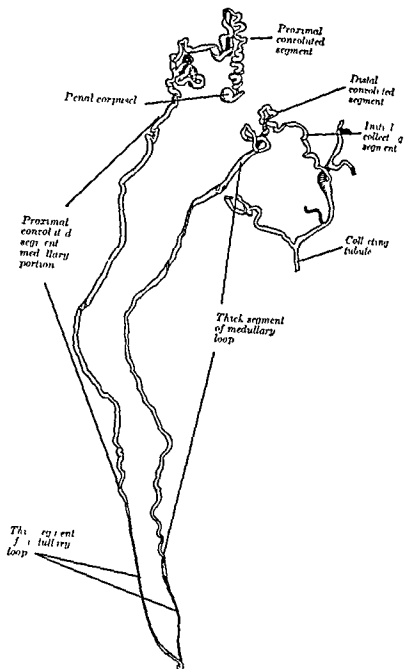


FIG. 180 — A complete renal unit (nephron) isolated by maceration from a normal kidney.  $\times 10$  (Redrawn from Oliver and Lund courtesy of Arch. Path.)

the abdominal cavity while visceral peritoneum covers the organs which extend into it. Both are backed by a delicate basement membrane as is indeed the entire tubule. Glomerular filtrate is drained away by a tubule leaving the renal corpuscle at a point generally remote from the vascular attachment of the glomerulus. The

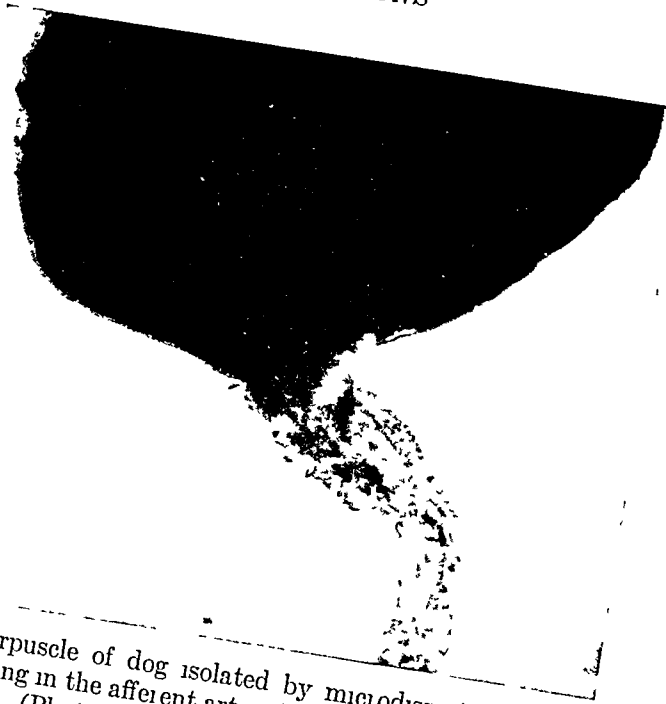


FIG 181 —Renal corpuscle of dog isolated by microdissection and stained with iron hematoxylin. The swelling in the afferent arteriole caused by the juxtaglomerular apparatus is clearly visible  $\times 450$  (Photomicrograph given by Dr Jean Oliver)

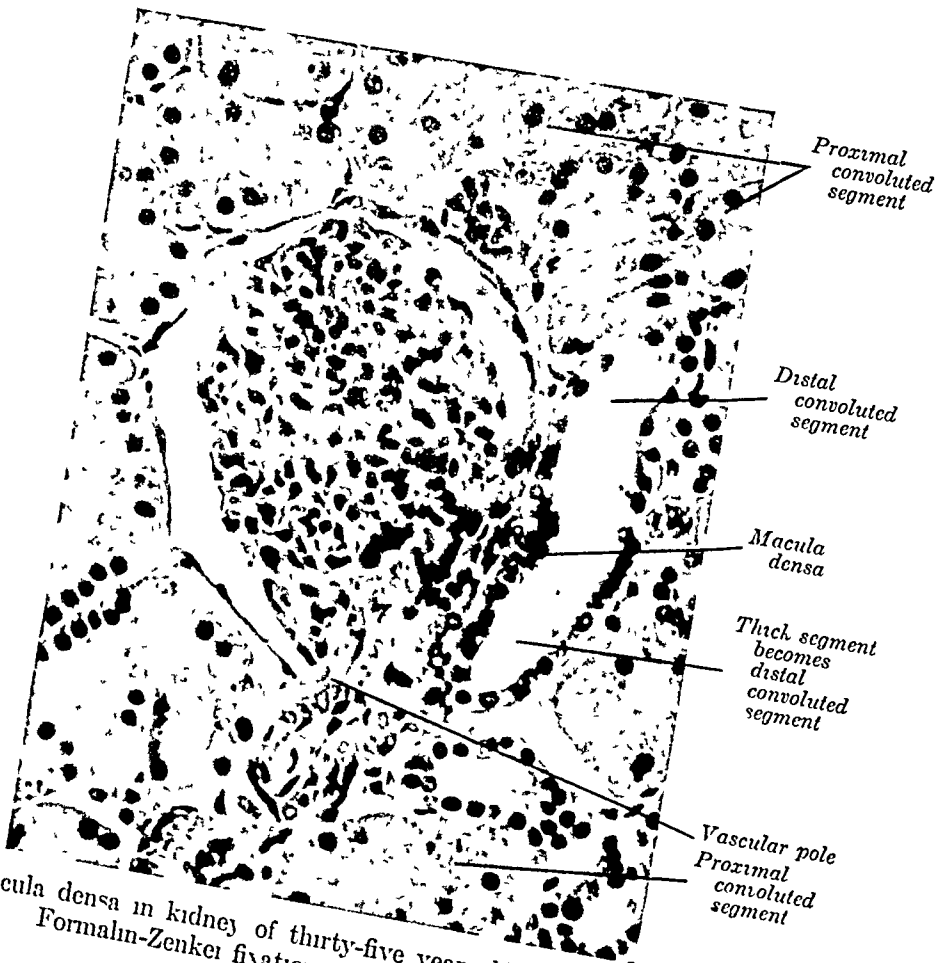


FIG 182 —Macula densa in kidney of thirty-five year old male executed Negro. Formalin-Zenker fixation and H & E  $\times 325$

urinary pole as contrasted with the vascular pole of the corpuscle (Fig 183). It is not correct to use the term glomerulus as synonymous with renal corpuscle of which it is simply a part. Renal corpuscles are also known as Malpighian corpuscles.

1. The *proximal convoluted segments* or tubules as they are frequently called usually lead off directly from the nephrons (Fig 183) but interposed between them and the nephrons may occur short neck segments made up of clear rather flat cells like those of Bowman's capsule. Identification of the proximal convoluted segments is easy. They are concentrated in the periphery of the lobules about the renal corpuscles and make up most of the bulk of the cortex of the kidney. They stain much more deeply with eosin than the occasional distal convoluted seg-

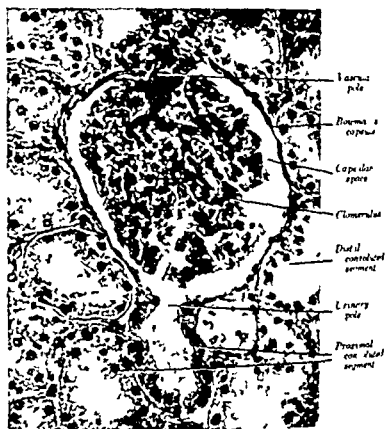


FIG 183. Renal corpuscle showing vascular and urinary poles. Same specimen as figure 182.  $\times 325$ .

and initial (or arched) collecting tubules found in this locality. This is partly because their epithelial cells have basal striations and brush borders. The former may be unusual mitochondria and the latter resemble slightly the cuticular lamellae of absorptive intestinal cells. Unfortunately the epithelium of this segment is particularly susceptible to postmortem changes so that in ordinary preparations the distal surfaces of these cells with their brush borders are often much broken up. They are however well shown in figure 184. These brush borders are limited to the proximal convoluted segment and to its medullary portion. Other distinctive structural features of this segment are (1) the great width of the cells in cross-section, of which few nuclei are seen in cross sections, (2) the indistinctness of cell walls, which are of very irregular shape (Foote and Grafflin, 1912), and its greater thick-

ness (50 to 60  $\mu$  outside diameter) By a special technique it can be demonstrated that alkaline phosphatase is present in large amounts (Kabat and Furth, 1941)

2 The comparatively straight *medullary portions* are structurally and functionally parts of the proximal convoluted segments of which they are the continuations in the medullary rays and outer parts of the medulla (Fig 184), but interesting evidence has been advanced of segmental differentiation in rats (Grafflin, 1942) Both can be beautifully demonstrated by vital staining with trypan blue

3 The *thin segments* are the parts of the medullary loops (of Henle) that usually extend farthest into the medulla No difficulty is experienced in their identification They are illustrated in longitudinal and cross sections (Figs 185 and 186) They are to be distinguished from large capillaries by the absence of blood in them and by the fact that their epithelial cells are less flattened than capillary endothelial cells so that more nuclei are included in cross sections In favorable specimens a few thin segments can be observed turning upward

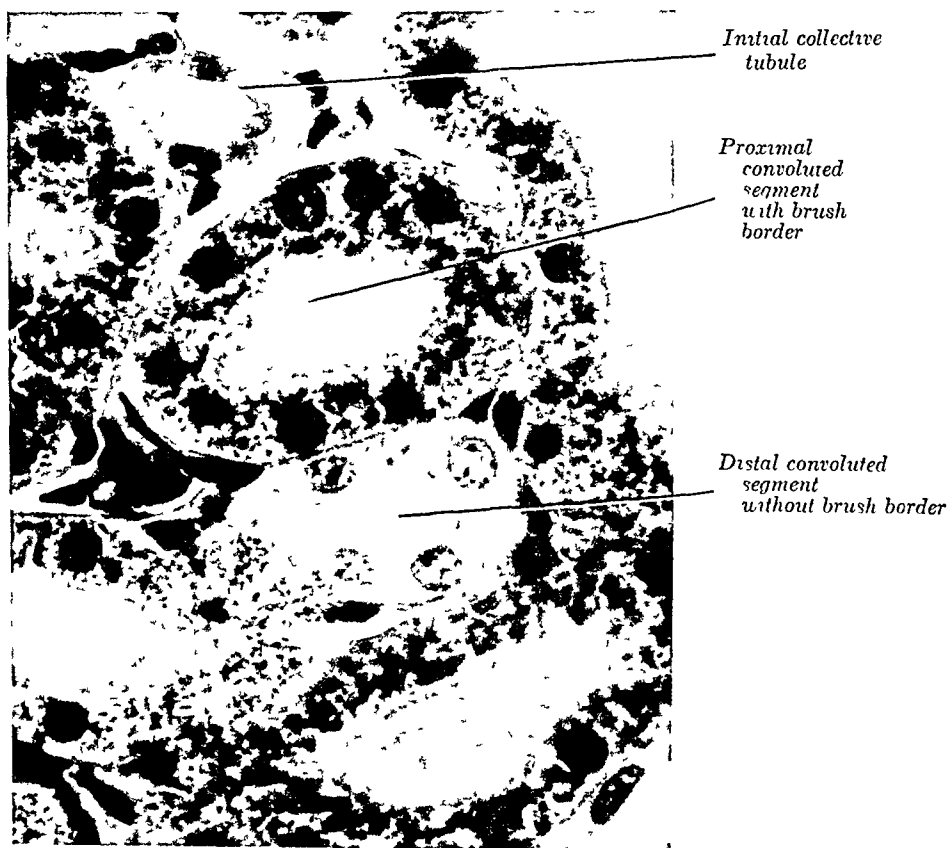


FIG 184 —Brush border, in kidney of five day old white male Same specimen as figure 178 Fixation in formalin-Zenker, iron hematoxylin stain  $\times 960$

4 The *thick segments* of the medullary loops are continuations upward of the thin segments Since they usually begin at points nearer to the renal pelvis than the places in the descending loop where the medullary portions of the proximal convoluted tubules transform into the thin segments (Fig 180), there are places in the medulla where they are present and the medullary portions are lacking Occasionally in the outer medulla, in sections cut parallel to the length of the tubules, loops of thick segments turning upward can be made out (Fig 185) These are



from renal corpuscles located in the outer cortex the thin segments of which occupy only the outer part of the descending loop. The thick segments ascend in the medulla and medullary rays pass out into the peripheral parts of the lobules and come into close contact with the afferent arterioles of their glomeruli (Fig. 152) where they become distal convoluted segments of which they are the straight portions.

5 The *distal convoluted segments* are much shorter than the proximal ones so that they are cut in fewer sections in the neighborhood of the renal corpuscles. Besides their relative infrequency of appearance in this region there are other points to be considered in distinguishing them from proximal convoluted segments. Their outside diameter is usually less, their cells are shorter, stain less intensely and are not equipped with brush borders. Moreover they are not so widely as

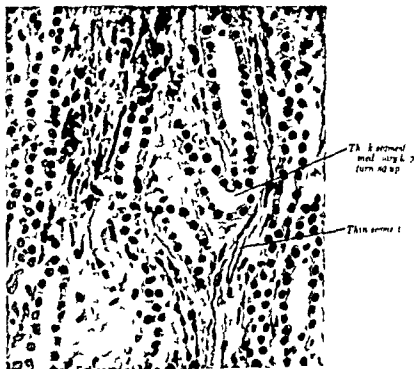


FIG. 153. Outer medulla of kidney of forty-six year old colored male who died in an accident. Autopsy thirty minutes later. Fixation formalin Zenker H & E.  $\times 300$  (U.S. Dept. of Pathology, Washington University, No. 10430, tissue given by Dr. R. F. Stowell)

the cells of the proximal convoluted segments so that nuclei are more numerous (Fig. 154)

6 The *initial collecting segments* pursue arched courses from the distal convoluted segment and enter collecting tubules in the medullary rays.

The collecting tubules, which thus receive urine from the nephrons pass toward the pelvis in the medullary rays and pyramids. They are of course less numerous in the medullary rays than the medullary portions of the proximal convoluted segments and the thick segments of the medullary loops because each serves more than one nephron. Contrasted with these parts of nephrons their walls have in H & E preparation a clear blue color not so tinged with red (Figs. 150, 151 & 153). Occasionally one finds two becoming confluent. Approaching the pelvis they enlarge and are given other quite unnecessary names.

It may be safely assumed that these several divisions of the nephron are nicely adjusted in size and structure to the function served. Division of labor among them has been under discussion for a long time. It is not advantageous to have the glomeruli exceed an optimum size, but the number is considerable. In both kidneys taken together there are from 2 to 8 million of them. The combined filtration surface is about 1.5 sq. meters (see accurate measurements for rats by Kirkman and Stowell, 1942). The complex plant carbohydrate, inulin, is filtered out by the glomeruli in the same concentration as that present in the blood and is neither excreted nor absorbed by the tubules. Consequently the volume of plasma, cleared of inulin per minute is the volume of the glomerular filtrate. The volume of filtrate in twenty-four hours is in the neighborhood of 150 to 200 liters. To let this escape would be both uneconomical and fatal. The purpose of conducting this great flood

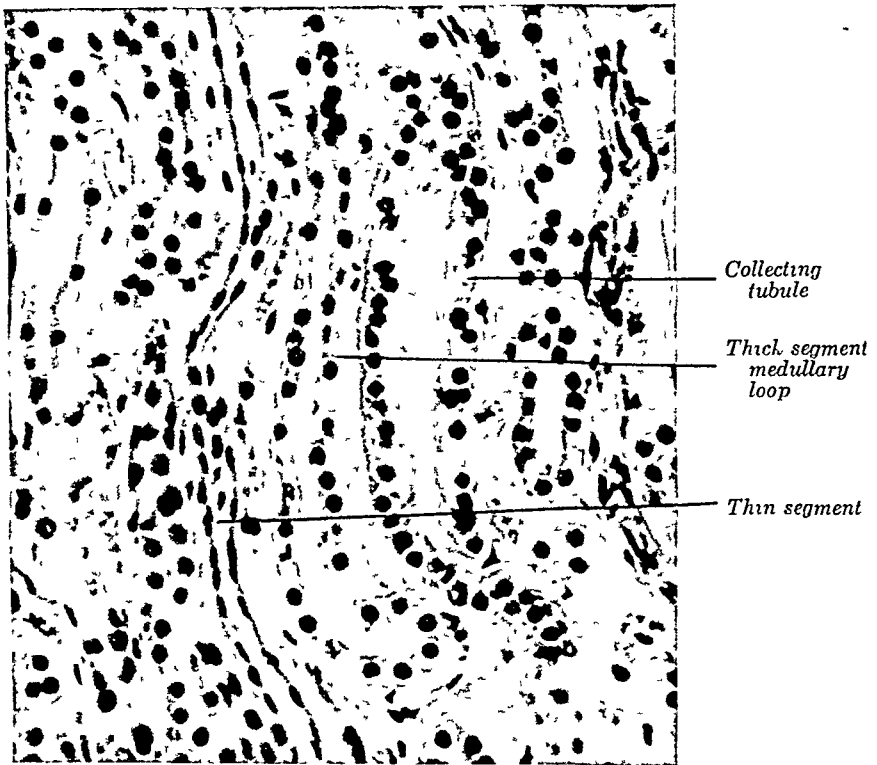


FIG 186 —Middle of medulla from same preparation as figure 185

through so many long, highly absorptive tubules is to recapture about 99 per cent of it, not in a haphazard way but selectively so that the components, which can be used over and over again, are retained while the waste products and the substances present in too high concentration in the blood are eliminated. A consequence of this tremendous backward movement of fluid is that the tissue fluid between the tubules is being flushed out mostly into the capillaries at the rate of over 100 cc per minute.

The researches of Richards and his associates on lower forms have been extended to the nephrons of mammals by Walker and Oliver (1941) and by Walker, Bolt, Oliver and MacDowell (1941). These investigators have made direct analyses of fluids from renal corpuscles, proximal and distal convoluted segments. The details we leave to experts, but it can be accepted as proved that all segments absorb

water and urea and that the proximal convoluted segment absorbs sugar, electrolytes and other substances. The distal convoluted segment absorbs base so that the urine becomes more acid.

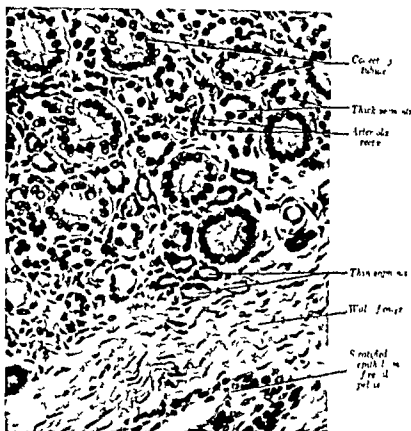


FIG. 187.—Cross section through side of renal pyramid. Same specimen as figure 1. Stained with H & E.  $\times 32$ .

Nerve endings can be demonstrated in the epithelial cells but their function is obscure. What is known concerning the hormonal control of the tubular epithelium

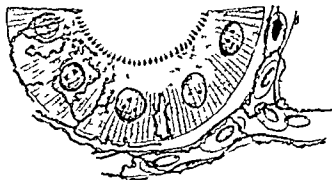


FIG. 188.—Types of nerve endings among the cells of a convoluted tubule of a kidney. Methylene blue. (Redrawn from Boeke in Penfield's *Neurology*, Paul H. Hoeber, Inc.)

is summarized by Shinnon (1942). The adrenal cortical and the pituitary antidiuretic hormones are somehow involved.

The origin of renin, a "pressor" substance produced by the kidney, which increases blood pressure, is not accurately known. Kaplan and Meyer (1942), in their examination of the kidneys of pig fetuses, find that the renin content is independent of the arterioglomerular component and directly dependent on the number, size, and functional state of the tubular component. The authors point to the absence of specialized juxtaglomerular cells (Fig 181) in these kidneys as indicating that cells of this sort are not essential to the manufacture of renin. The *macula densa* (Fig 182) has also been mentioned in this connection. The source of an "antipressor" substance that reduces blood pressure is obscure.

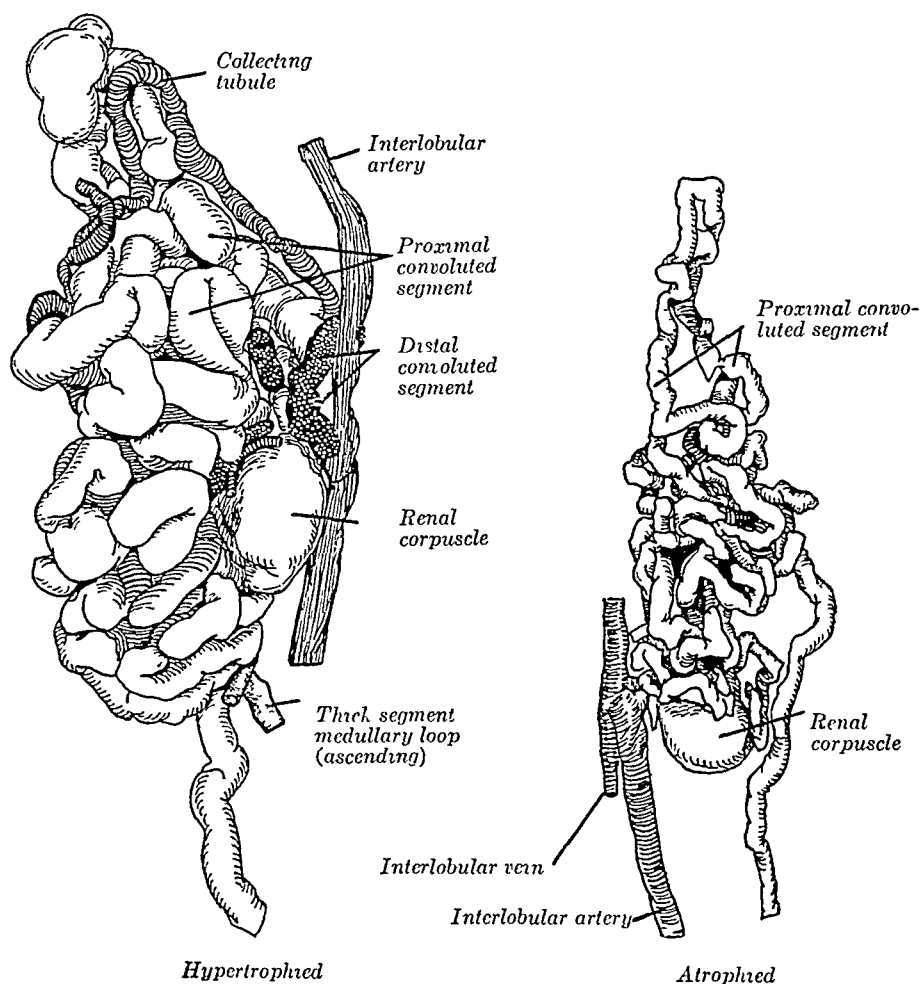


FIG 189 —Reconstruction of hypertrophied and atrophied renal unit to show range in size in chronic Bright's disease  $\times 50$  (Redrawn from Oliver and Lund, courtesy of Arch Path)

Like so many other organs, the kidneys have a large *margin of safety*. Urine formation is adequate after one has been removed. It may be concluded from the fact that cell division is seldom observed that replacement is normally not a very conspicuous process. Renal epithelial cells are probably rather long-lived. Oliver (1933, 1934) attributes the tenacious survival of patients suffering from Bright's disease to certain patchy areas of tubular hypertrophy. He investigated alterations in size and shape by making reconstructions from serial sections and by the maceration in acid and isolation of individual tubules by careful dissection in much the

same way that Huber investigated normal kidneys some thirty years ago. Oliver discovered that the outstanding modification is in the proximal convoluted tubule which may hypertrophy as much as fifteen times chiefly by increase in length and thus replace in physical size 15 destroyed tubules. Figure 189 gives a concept of the range in size between hypertrophy and atrophy. He suggests that it may be on this portion of the renal unit that the slowly disintegrating organ gradually relies more and more. Another significant observation was that tubular integrity does not depend upon a normal glomerulus. Some hypertrophied tubules had glomeruli which were reduced to bloodless masses of collagen and could therefore be regarded as physiologically aglomerular (Fig 190). Yet from the histological appearance of the cells of such an hypertrophied tubule there is no reason to doubt its functional ability so that it is analogous in a way to the aglomerular

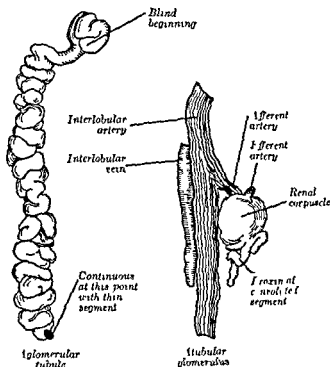


FIG 190 Reconstructions of aglomerular tubule and atubular glomerulus from case of hemorrhagic Bright's disease  $\times 50$  (Redrawn from Oliver and Lund courtesy of Arch. Path.)

tubules of certain fishes. In some contracted kidneys the aglomerular tubules were even more numerous than the glomerular ones. And Oliver also found atubular glomeruli (Fig 190) the functional service of which, if any, challenges the imagination.

**Urinary Passages** - From the minor renal calyces that cap the pyramids to including most of the urethra the urine passes through channels different from the segments of the nephrons and the collecting tubules in two particulars. Their epithelial lining is stratified consists of several layers of cells closely fitted together and is much less permeable. The muscles in their walls provide both the propulsion of fluid and for its arrest at strategic points by sphincter action.

The minor calyces are hollow muscular cups that fit snugly over the apices of

the pyramids and lead through slightly narrowed stems into the renal pelvis. By slow, wave-like contractions, beginning proximally and proceeding distally, they are thought to press the urine out of the pyramids into the renal pelvis as one presses the milk in streams out of the teats of a cow. The walls of the pelvis itself are also contractile and force the urine into the funnel-shaped opening of the ureter. How far this muscular action is required is debatable, for the kidney can maintain a fairly high excretion pressure so that the urine would naturally pass in the direction of least resistance.

The *ureters* are long narrow tubes, the muscle of which is disposed in two chief layers—inner longitudinal and outer circular—the reverse of the arrangement in the digestive tract. Urine is encouraged to flow onward by rhythmic peristaltic contractions. A good description of nerve supply is given by L. R. Wharton (1932). Since the ureters pierce the wall of the bladder at an angle, their lumina are compressed when the bladder is distended.

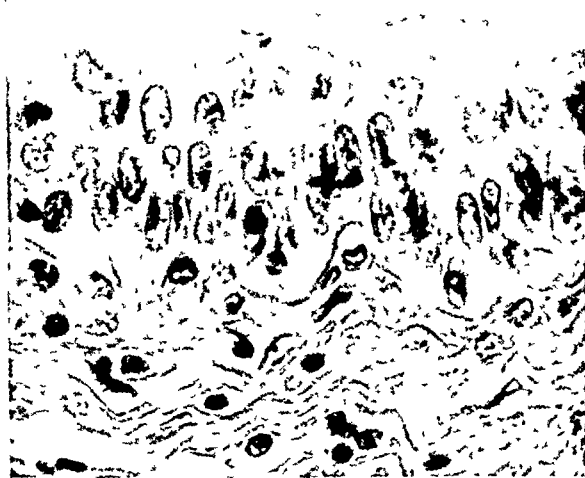


FIG. 191 —Urinary bladder to show transitional epithelium

Obviously the *bladder* is a urinary reservoir. There are three layers of muscle—inner longitudinal, middle circular and outer longitudinal—but these are not sharply defined except at the internal sphincter guarding the exit of the urethra. A well-developed intrinsic nervous mechanism exists in the wall (Iljina and Lawrentjew, 1932) and search should be made for plexuses like the submucous and myentericus of the intestine. The autonomic nerve supply is described by Gruber (1933). Elastic fibers are abundant. These early show the signs of ageing. The epithelium of the bladder (Fig. 191), as well as that of the renal pelvis, ureters and prostatic urethra, is of a special type, designated “transitional,” not found elsewhere. The cells next the lumen are large and have a distinctive bloated appearance.

The prostatic urethra receives drainage from the prostate (Fig. 271). The membranous urethra and the female urethra possess occasional intra-epithelial nests of mucous cells and some small urethral glands (of Littre). Both are guarded by sphincters of striated muscle. For the nervous control of micturition see Cloake, Learmouth and Barrington (1931) also, on the clinical side, Langworthy *et al* (1936). The most detailed and best illustrated account of the entire urinary system is provided by V. Mollendorf (1930).

## SUMMARY

The kidneys do more than maintain the constancy of the salts in the tissues. They are the leading regulators of water metabolism (Peters 1942) excrete metabolic products especially nitrogen and the sex hormones. Each kidney is made up of a million or more nephrons which may be regarded as the functional units and each of these is divisible into a renal corpuscle, proximal convoluted segment with medullary portion, thin and thick segments of the medullary loop, distal convoluted and initial connecting segments. The renal corpuscle looks like a blood filter. It consists of capillary loops invested by a delicate layer of epithelium constituting a glomerulus, enclosed in a capsule from which the proximal convoluted segment leads. The circulation favors the glomeruli since all the arterial blood traverses them before it supplies the renal tissue. For nearly one hundred years the part played by the glomeruli and the various segments of the tubule in urine formation have been actively debated. Now it is clear that glomerular filtration is produced in large volume and that much of this is selectively reabsorbed by the segments of the nephron. Renal function is largely endocrine controlled. The urinary passages that conduct the urine to the outside are epithelial tubes generally provided with muscle so that here nervous regulation looms large.

## CHAPTER XIV

### NERVOUS SYSTEM

THE nervous system also integrates, but in quite a different way from the blood stream, not by providing the essential medium, water, and by water-borne transportation of materials but by impulses that are rushed along nerve fibers at a speed of about 87 miles per hour. The vital unit is the nerve cell, but the integrative unit is a reflex arc like the one illustrated in figure 192. The stimulus is received in a receptor (the skin), the impulse is passed centrally by an afferent conductor (a sensory cell), it is adjusted in the center (perhaps suppressed or strengthened) and an efferent conductor (a motor cell) transmits it to an effector (in this case muscle). The actual connections are of almost unbelievable complexity. These we cheerfully leave to the course on neuroanatomy, for which there are several excellent textbooks, but a few facts of general interest must be mentioned to round out presentation of microscopic form and function in the human body

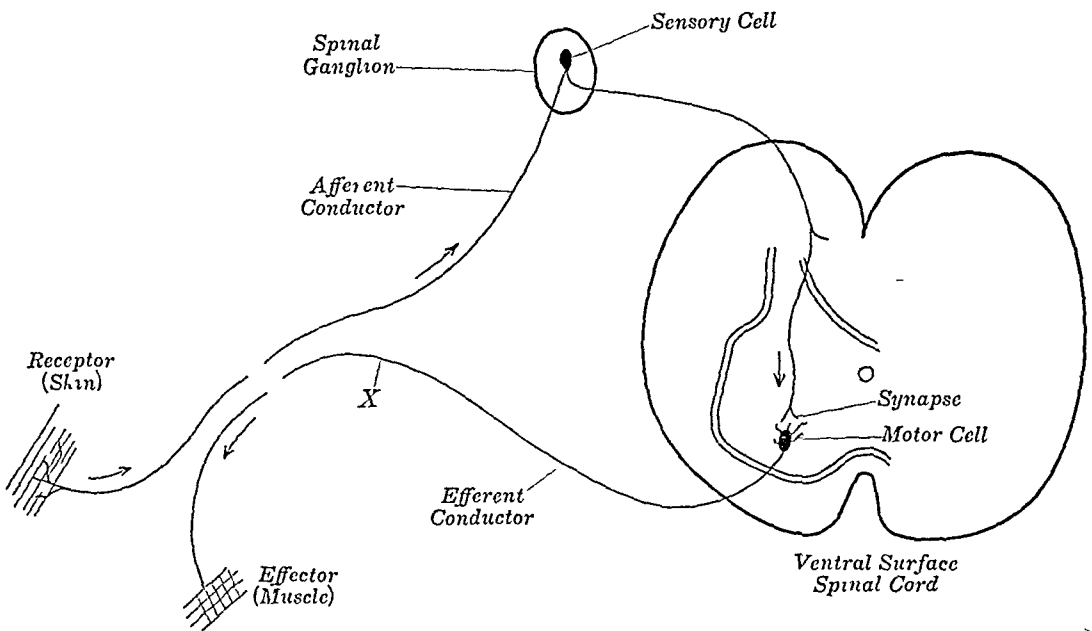


FIG 192 —Diagram of simple reflex arc involving skin, spinal cord and striated muscle.

**Nerve Cells.**—Gland cells respond by secretion, muscle cells by contraction and nerve cells by conduction when adequately stimulated. The stimulus is received from cells so constructed and located as to be activated by changes that evoke no response in other cells—in other words their responsiveness to certain stimuli is very great while to others it is but slight. The chemoreceptors for taste and smell have been mentioned. The receptors in the eye and ear will be briefly described later and some of those in the skin in Chapter XV.

The shape of nerve cells is bewildering in its variety, but when each type is examined it is found to be adjusted to the performance of a special task. Some of them are the longest and the thinnest of living elements, while others are quite short. All of them are divisible into cell body and conductile processes. The body



contains the nucleus and is always the widest part. Sometimes the largest of bodies give rise to the longest processes. Thus a motor cell of the anterior horn of the spinal cord may have a diameter of about  $50\ \mu$  and extend an axon (G. *axis*)  $7\ \mu$  in diameter to the muscles of the foot a distance of approximately 1 meter. It is true that we cannot follow an individual axon by actual dissection all the distance but we know that when it is cut off from the cell body, say at the point A in the diagram it degenerates distally throughout its length and the degeneration reaches to the muscle. A simple calculation shows that the relation of diameter to length is the same as that of a telephone wire 2 mm in diameter, extending 20 kilometers. This single efferent process can be distinguished from the afferent ones, or dendrites (G. *dendrites* tree-like) which gather the impulses into the cell by the examination of almost any section of the spinal cord. The cell body is identified by its single large spherical nucleus and surrounding cytoplasm. In the latter basophilic Nissl bodies may be seen. These extend a short distance out into the dendrites which are branching processes of diminishing girth but are never found in the axon or in the cytoplasm from which the axon springs (axon hillock). Such a motor cell as one in this picture in its entirety is illustrated in figure 193.

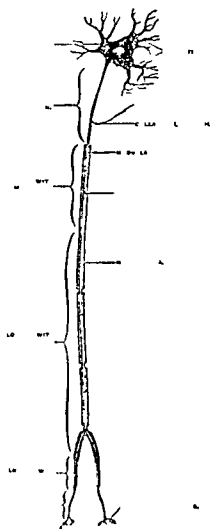


FIG. 193 —A nerve cell (Stöhr)

The sensory cells of the spinal ganglia are just as long and equally impossible to study as isolated individuals. In their case the peripheral process may be 1 meter or more in length carrying impulses inward. A short distance from the cell it becomes confluent with the central process which transmits into the cord. Examination of spinal ganglion sections shows globular cells with very few processes. Often each cell has only one process which the Nissl bodies do not penetrate

and which is termed a dendraxone because it gives rise to two—one conduct toward the cell and the other away from it.

The bodies of both large and small nerve cells can be studied in *beds* in thick sections by a method which Golgi discovered almost by chance and which bears his name. It consists of soaking the tissue in potassium bichromate solution and subsequent infiltration with silver nitrate. The resulting preparations lack the failures until they are examined in detail when in some areas a few individual nerve cells are found to be blackened with all their processes though the majority are unaffected. The reason why some should be picked out remains a mystery. If

were not so, if all were completely impregnated, they would hide each other so effectively that none of them would be fully visible. The appearance of a few cells is represented in figure 194. The length is proportionally much greater than that indicated. Actually, the length of the axone of the motor cell (C) is about 60,000 times the diameter of its cell body, whereas in the diagram it is only approximately 100 times. The portrayal of the short cells (B) and (D) is more accurate but is not to scale.

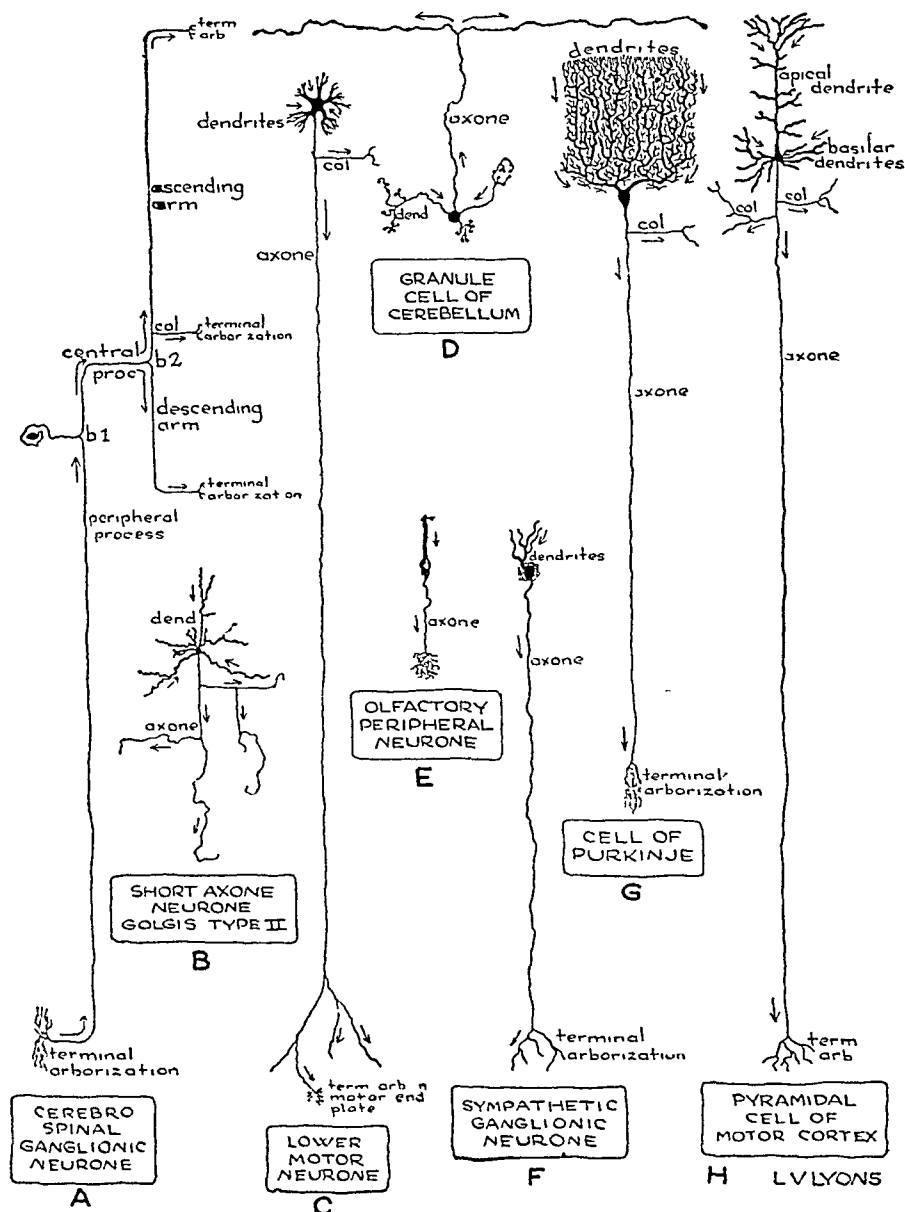


FIG 194 —Some of the principal forms of nerve cells. The direction of conduction is shown by the arrows. (Bailey, Textbook of Histology, Williams & Wilkins Company.)

Obviously, nerve cells must maintain their shapeliness. The surface film may consist of monomolecular layer of protein or of lipid, but it possesses a microscopically visible thickness and definite tensile strength so that we may safely assume that it is reinforced. The question is, how? Reference again to a section of a spinal ganglion will show that the nerve cells are invested by special capsule

cells. Some long axones are encased in sheaths which vary in thickness depending on their type and the particular part of them examined. The fibers are held together by connective tissue as individual telephone or telegraph wires are held together in a cable. Nerve fibers are so strong and flexible that they are used for thread by certain tribes in the North. They are of whitish color, owing to their vascularity.

In ordinary H & E stained sections nerves can often be identified. They frequently occur beside blood vessels. Seen in *cross sections* the outline of a nerve is usually rounded. It is invested with a connective tissue sheath, the *perineurium*—the collagenic fibers in which are disposed in two decussating spirals (Glees, 1914). Septa of perineurium may extend into the nerve and separate the nerve fibers.

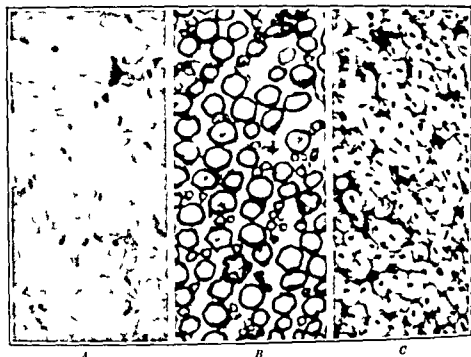


FIG. 19.—Cross sections of cat sciatic nerves prepared by different techniques. A, 10 per cent formalin, hematoxylin and eosin. B, 1 per cent osmic acid. C, Run on a pyridine silver. A shows but little. In B the sheaths of myelinated fibers appear black. In C the axones of both myelinated and non myelinated fibers are impregnated.  $\times 400$ . (Photomicrograph by J. I. O'Leary.)

bundles. About the individual fibers are minor sheaths which make up the *neurilemma*, likewise consisting of vascularized connective tissue. The medullary or *myelin* sheaths appear as faintly staining thick walled circles (Fig. 19, A). From each myelinated fiber is a very thin sheath, the *neurilemma* (G. *neurion* + *lemma*, but k) or sheath of Schwann, which unlike the endoneurium and perineurium is of ectodermal origin. It is made up of a single layer of flattened cells, the cytoplasm of which is hardly distinguishable in specimens of this sort although the nucleus of an occasional Schwann cell can be made out closely applied to the myelin sheath. In such cross sections the myelin sheaths are seen to be distended to accommodate the nuclei of Schwann cells. Treatment with osmic acid demonstrates the myelin sheaths more sharply (Fig. 19, B) and shows the nerve

their size, while silver impregnation reveals the shrunken axones of both myelinated and non-myelinated fibers, the sheaths remaining uncolored (Fig. 195, C)

When nerves are examined at acute angles, or longitudinally, in routine preparations these features are not so readily observed. Figure 196 is a diagram of two single myelinated fibers. The myelin sheath is blackened and is discontinuous at fairly evenly spaced intervals, the *nodes of Ranvier*. Between each node the neurilemma (*a*) consists of the cytoplasm of a single Schwann cell of which the nucleus is shown (*c*)

Figure 197 is particularly instructive because it shows the appearance of the same isolated myelinated fibers when examined in polarized and ordinary light. In the former the myelin sheaths shine out brightly and it is evident that they have cleftlike cuts, the *incisures* of Schmidt-Lantermann

Figure 198 illustrates a partly longitudinal action of an eye muscle nerve in which these incisures are very conspicuous. By microdissection de Renyi (1932) has found that the neurilemma can be easily detached from the myelin sheath. At the nodes of Ranvier, where the myelin sheath is interrupted, the neurilemma comes into direct contact with the axone in peripheral nerves and is adherent to it. Where present the myelin sheath is closely connected with the underlying axone. By x-ray diffraction studies Schmitt *et al* (1941) have investigated the molecular structure of myelin sheaths. They are constructed of "concentrically wrapped layers of mixed lipides alternating with thin, possibly unimolecular layers of neurokeratinogenic protein material"

The cytoplasm of nerve cells is fundamentally similar to that of other cells. As is to be expected it contains mitochondria (Fig 199). Nissl bodies are present in the cell body, and larger dendrites, but are absent in the axone and the part of the cytoplasm from which it springs (axone hillock). They stain deeply with basic dyes like methylene blue, exhibit quite distinctive patterns of arrangement in some cell types (Malone, 1932) and are regularly modified by injury to the cell (Fig 200). Tinctorially, and probably chemically, they resemble the basophilic material in the proximal parts of acinous cells of the pancreas. Another fundamental cytoplasmic component is the Golgi apparatus, which, here as elsewhere, shows rather definite responses to injury (Fig 201).

Very striking in special preparations, but somewhat more elusive are the neurofibrils (Fig 203). In their interpretation we must remember that fibrillar formations are not unusual in other cells (epidermal, muscular, etc.). Covell and Scott (1928)

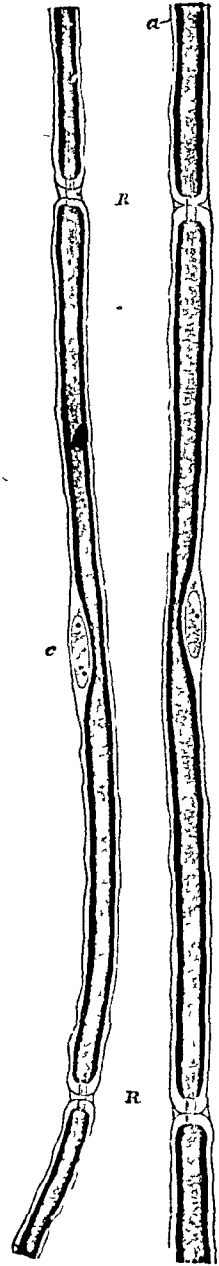


FIG 196—Diagram of myelinated nerve fibers treated with osmic acid. R, Nodes of Ranvier, a, neurilemma, c, nucleus (Gray's Anatomy, after Schaffer)

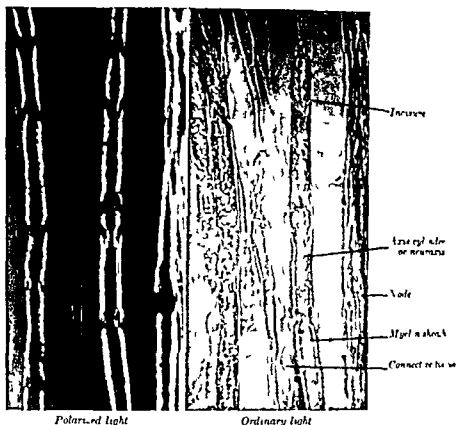


FIG. 197 --Isolated peripheral nerve fibers of rats in chronic vitamin B<sub>12</sub> deficiency viewed with crossed Nicols (polarized light) and with direct illumination  $\times 500$  (From Prickett Salmon and Schrader courtesy of Am. J. Path.)



FIG. 198. Section of nerve to eye muscle of male aged forty-five years with melanoma of conjunctiva fixation and H & E

have furnished strong experimental evidence that the Golgi apparatus or network results from the action of the technique on droplets of material stainable with neutral red in freshly teased nerve cells as illustrated in figure 202. Nos. 5—8 of this figure show the blackening of the red stained granules with osmic acid and the

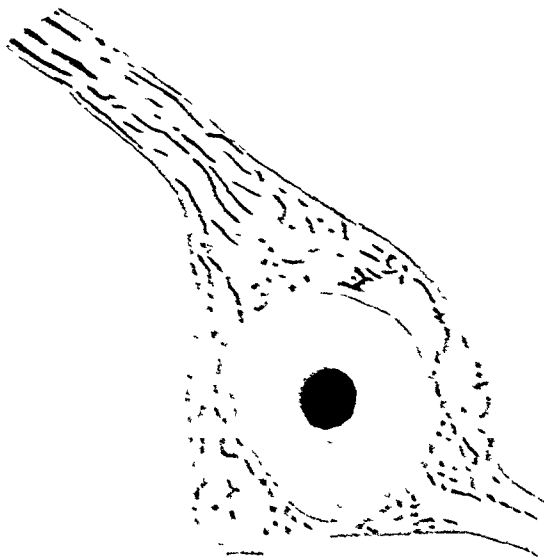


FIG. 199.—Large nerve cell of white mouse in which filamentous and red-like mitochondria have been stained by Cowdry's method.  $\times 1600$ . (Nicholson, *Am. J. Anat.*)

arrangement of a few of them in linear series. Nos. 9—11 illustrate the affinity of similar granules for silver. No. 12 is a better instance of the linear arrangement of the neutral red granules. 13 an incomplete and 14 a complete impregnation with

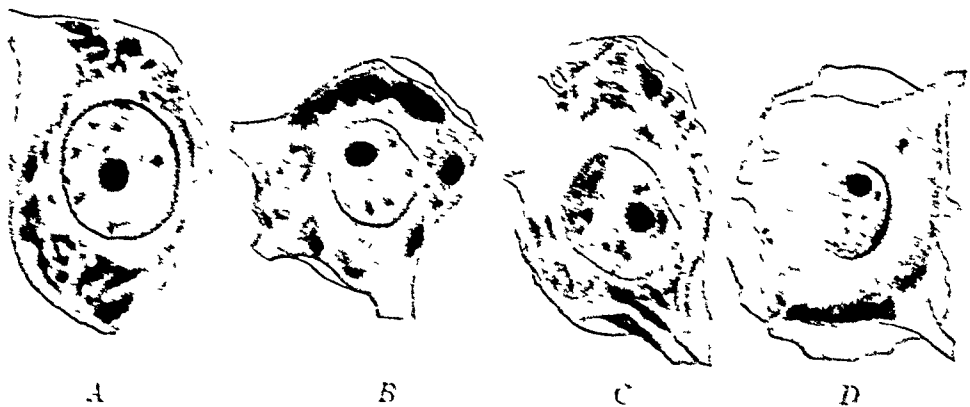


FIG. 200.—Stages in the disappearance of iron-containing protein, which corresponds at least partly to the Nissl substance, in the cells of the hypoglossal nerve of the white rat following axon injury: A, The normal; B, the third day; C, the seventh day; D, the fifteenth day. (Nicholson from Cowdry, *General Cytology*, University of Chicago Press.)

osmic acid. Such preparations demonstrate that, under the influence of the technique, the granules become arranged in series, take up osmium or silver, as the case may be, and become confluent to form a typical Golgi network. This conclusion is supported by the observations of Cowdry and Scott (1928) on the genesis

of the Golgi apparatus in the malarial parasite, in which every stage in the transformation can be followed under the microscope.

Of these four components only the mitochondria are clearly visible in living nerve cells but the regularity of the occurrence of the others in fixed cell and the significant modifications that they undergo in different functional states evidence that they are not altogether artifacts. Bensley and Gersh (1933) employing a re-



FIG. 201.—Response of the Golgi apparatus of the cat. A Normal anterior horn cells. B Reticulation in an anterior horn cell seven days after section of the sciatic nerve. C Reticulation and resolution in a cell of Clarke's column four days after section of the cord (Cowdry after Penfield, in Penfield's *Neurology*, Paul B. Hoeber, Inc.)

refined technique involving freezing in liquid air and dehydration in vacuo while still frozen thus avoiding chemical fixation discovered traces of Nissl bodies but not of neurofibrils though the latter do occur and can be seen in certain living invertebrate nerve cells. Cellular mineral ash is represented in figure 204. Lipoid granules are present also pigment in some cases and enzymes, but as far as is now known the chemical components of the cytoplasm are not significantly different from those of other cells though they probably are present in different proportions.

#### LEGEND FOR FIG. 202

FIG. 202.—Nos. 5-8 are camera lucida drawings of the same cell from a segment of the spinal cord of a vitally stained mouse (5) before (6) ten minutes (7) seventeen minutes (8) seventy five hours after treatment with osmic acid. Nos. 9-11 A similar experiment to determine the effect of Da Fano's silver technique on the neutral red granules. (9) Fixation (10) three minutes after addition of fixative containing cobalt nitrate and (11) after twenty minutes fixation one hour silver nitrate fifteen minutes reduction in Fehling's mixture. No. 12 A vitally stained cell thirty minutes after fixation in Kolmer's fluid. The vacuoles have coalesced to form tortuous channels and the granules are arranged in single file. No. 13 An incomplete and complete osmic acid impregnation of two adjacent cells. (From Covell and Scott courtesy of Anat. Rec.)

## NERVE CELLS



FIG 202



The nuclei of large nerve cells are characterized by one or more prominent nucleoli and by the empty appearance of their nucleoplasm compared with the marked basophilia of their cytoplasm. Herrick (1927) believes that activity affects

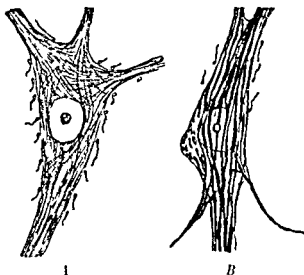


FIG. 203.—Functional changes in neurofibril in a hibernating animal. A during activity; B during rest. (Cowdry, after Cajal, in Penfield's *Neurology*; Paul B. Hoerber, Inc.)

the explosive type and that this quality is largely dependent on the presence of much chromophilic substance (Nissl bodies) which is presumably oxidizable, thus throwing out its entire mass almost instantaneously—in other words, that “the organization of protoplasm of the nerve cell is such that a very small stimulus will liberate a large amount of energy with explosive suddenness.”



FIG. 204.—Part of cerebellum of cat fixed in absolute alcohol-formalin mixture cut in sections  $4\mu$  thick incinerated, viewed in the dark field and photographed. Light is reflected from the mineral salt, the background being dark. The mineral skeletons of two Purkinje cells are shown. The one on the right is marked by the dense heavy white salt of the nucleolus surrounded by nucleoplasm relatively free from salt. The cytoplasm about the nucleus exhibits a good deal of salt, some of which may have been derived from Nissl bodies.  $\times 750$ . (Photomicrograph by Gordon H. Scott.)

**Dynamic Polarization**—This is present in all gland cells that have receiving (proximal) and discharging (distal) poles—is very highly developed in nerve cells. The impulse is usually received by the dendrites and is passed on through the axone. Linkage of cells in series involves its passage across the *synapse* (Greek *syn*—binding together) where one axon ends and the dendrites of the next cell pick it up. Structural features of the synapse are described by Bartholmez and Hoerr (1933). The association is so intimate that at the point of contact it is not possible microscopically to demonstrate two meeting cells. But the physicochemical character of the material intervening between the two cells is of great importance. It is polarized so that the impulse passes

across in one direction only, and it serves as a protection for either cell can die while the other goes on living.

According to evidence critically evaluated by Danielli (1942) the essential component in the walls of all cells is the plasma membrane. This conditions permeability and its integrity is essential to cell life. It is said to consist of a continuous layer of lipid molecules (phosphatids, sterols, fats) not more than 2 to 4 molecules thick on which proteins are adsorbed. The lipoids give permeability and the proteins mechanical strength. In his opinion it is improbable that the lipid layer is ever thicker than  $10\text{ m}\mu$  and that the whole membrane is between  $1\ \mu$  and  $1\text{ m}\mu$  ( $=0.001\ \mu$ ) thick. We cannot, therefore, expect regularly to visualize the plasma membrane microscopically by visible light because the limit of visibility is a particle about  $0.25\ \mu$  in diameter. However, the location of the plasma membrane is made clear by the difference in properties of the cytoplasm which it limits and the surrounding tissue fluid and also, in the dark field, by light reflected from its surface.

Such a plasma membrane is obviously conditioned by the cytoplasm within and the fluid without. Where, as in a synapse, the plasma membrane of another cell comes into very close contact with it one would not expect its properties to remain the same, though each plasma membrane continues to guard the life of the cell to which it belongs. Another modifying factor may be the circumstance that the giving cytoplasm on one side is axonic while the receiving cytoplasm on the other is not axonic but belongs to dendrites or cell body. There is reason to think that the two are not the same so that a one way differential is established which may underly the polarization. A perennial subject of debate is the permanence of these synaptic connections. Support is given to the idea that the intimacy of the association may change by direct observations of movements in living nerve endings made by Speidel (1940-41). On the whole it is unsafe to assume that the synapse consists of a single membrane. Other kinds of cells are often so closely pressed together that it is optically impossible to distinguish two plasma membranes yet two are present at least potentially.

Nerve cells are arranged in communication lines in accordance with several principles. Each synaptic connection means delay like the individual connections required in long distance telephone calls. In rapid long distance communication from spinal cord to muscles of the toe a single nerve cell is involved. Impulses from many sources converge and travel a final common path to the effector as telephone calls from many places finally come in on a single wire. Amplification is provided by one cell activating many. The message can be suppressed by inhibition. When one touches a hot stove the normal response is to withdraw the hand, but, by effort of will, this can be overruled.

Labor relations among nerve cells are quite different from those in most other parts of the body. In the endocrines, for example, each cell begins the manufacture of a product and carries it through to completion. There is no construction line in which a series of cells serve each carrying the job a little farther. And a factor of safety is always provided in the form of an excess of workers among whom the work is fairly evenly spread. Consequently a sit down strike is not on the cards. It is interesting to figure out how widespread is this arrangement in other parts of the body. In the nervous system a similar factor of safety exists in the presence of more nerve cells of every type than is generally essential for life of the whole community, but, if, in the lines of communication, one group ceases operations those

further along are frustrated and can no longer serve since they receive no impulse to pass on.

The replacement of worn out nerve cells is not feasible because after one year of age all of them have become so highly specialized that multiplication is impossible. They are in a word fixed postmitotics like neutrophile leucocytes (p. 23). But unlike leucocytes there is no reservoir of vegetative intermitotics from which others can gradually be evolved through a series of differentiating intermitotics. This mechanism of filling the gaps has differentiated itself out of existence. These are the list of the Molucans.



Thirty-two year white male  
killed in accident

Seventy-two year white male  
pulmonary embolism

Fig. 20. Lurking cells of young and old persons photographed in  $7\mu$  sections of tissue fixed in 10 per cent formalin and stained with methylene blue.  $\times 1000$  (Photomicrographs given by Dr. Warren Andrew)

Consequently it is essential for nerve cells to be long lived and to be protected so that a sufficient number will endure and supply rapid integration as long as the cellular community persists. It has been estimated that nerve cells live in man 2000 times as long as neutrophils in man (Cowdry, 1942). The life of neutrophils is however very hazardous. Heart muscle cells are as long lived as nerve cells, like them I say put. As yet no feature in the structural organization of a cell has been discovered which conditions length of life. Nerve cells as they reach the end

of their allotted life span, begin to show signs of senility (shrinkage, decrease in Nissl bodies, etc.) as indicated in figure 205. Because no new cells can take their place repair of injury is limited to the outgrowth of new processes from otherwise intact cells. This whole problem is reviewed by Young (1942). See also Bodian (1943).

The protection afforded cells differs with the part of the nervous system involved. For the cells of the peripheral and central nervous system it is not the same. Among the former, the tissue fluid environments of the nerve cells of sympathetic ganglia, and their liability to injury, are not very different from those of neighboring cells of other sorts. But nerve cells in the central nervous system are well protected by stout bony walls, held in place by connective tissue (neuroglia), and by membranes, and cushioned by a special tissue fluid.

**Neuroglia.**—The central nervous system has a type of connective tissue all its own, developed from ectoderm and called neuroglia (*G. neuron, nerve + glia, glue*) to indicate its binding properties. In addition, small mesodermal elements, akin to the reticulo-endothelial cells, invade the nervous system with the growing blood vessels. These are called *microglia*. Physiologists and pathologists have been slow to appreciate the significance and importance of neuroglia and microglia because most of the important discoveries relating to them have been achieved by the use of new and refined methods of silver impregnation which can be applied and interpreted only by experts. Another detriment is the complicated nomenclature. Suffice it to say here that neuroglial cells are to be differentiated from nerve cells in a negative way by the absence in them of Nissl bodies and the fact that their processes are short, tapering and never myelinated. The nuclei of the oligodendrocytes (*G. oligos, little + dendron, tree + kytos, cell*) look naked.

**Membranes.**—The first and outermost membrane is thick, hence the name, *pachymeninx* (*G. pachys, thick + meninx, membrane*). It is also called the *dura mater* (*L. hard mother*) and is represented in figure 206. The dura is separated from the underlying arachnoid membrane by a very narrow fluid-containing cleft encircling the brain, the subdural space, which is a closed cavity lined by a condensation of mesenchyme. The *arachnoid membrane* is also mesenchymal but is much thinner. It bridges over the sulci and limits externally a series of communicating subarachnoid spaces containing a good deal of fluid—considerably more during life than is seen in a brain after removal from the skull. The membrane is connected across the spaces with the innermost *pia mater* (*L. tender mother*) by cobweb-like strands so that the name, *pia arachnoid* (*G. arachne, cobweb + eidos, resembling*), is descriptive. The delicacy of the pia arachnoid and pia mater evidently impressed the older anatomists for the two were grouped together under the heading of lepto-

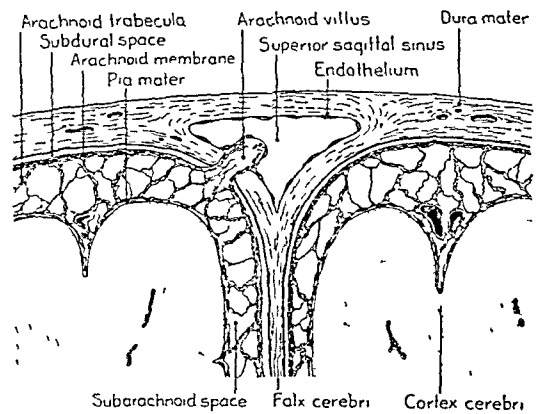


FIG. 206.—Schematic diagram of a coronal section of the meninges and the cerebral cortex, showing the relation of an arachnoid villus to the dural venous sinus. Weed, *Am J Anat*, courtesy of Wistar (Institute).

meninges (*G leptos* delicate + *meninges*, membranes) The pia mater is closely adherent to the surface of the brain and even follows the vessels into it (Fig 207)

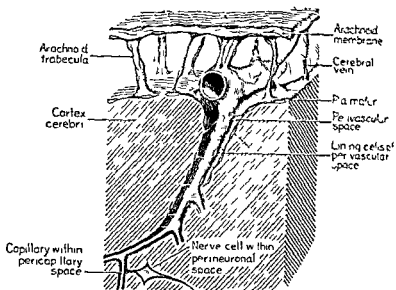


FIG 207 —Schematic diagram of leptomeninges and nervous tissue showing the relations of the subarachnoid space, perivascular channels and nerve cells (Weed, *Am J Anat.*, courtesy of Wistar Institute)

**Cerebrospinal Fluid** —The cerebrospinal fluid includes not only the fluid in the subdural and subarachnoid spaces but also that within the ventricles and in the central canal of the spinal cord. The shifting of this fluid with changes in posture, respiration and so on has been investigated radiologically by Reitan (1941). A good review of basic factors is that of Wislocki (1932). Normally the fluid amounts to about 50 to 100 cc., of a specific gravity of about 1.007 and contains inorganic salts, dextrose, some protein and a few cells. It is a more watery fluid than any other in the body except perhaps the aqueous humor of the eye. The cerebrospinal fluid comes from the blood stream and returns to the blood stream. We may regard the bony walls of the brain and cord as constituting a closed cavity into which nutrient arteries penetrate through various foramina. They pass through the membranes and spaces referred to, give oxygen and food to the nerve cells and meander as capillaries through the choroid plexuses (*G choroides*, skin like) which are special infoldings of parts of the thin roofs of the ventricles. In them the blood plasma is only separated from the ventricular fluid by endothelium, a thin film of tissue fluid and a single sheet of cuboidal epithelial cells. The rate of flow is slowed down owing to the increase in combined cross-sectional area caused by expansion into a capillary plexus. The emerging blood is collected in the choroid veins. It is generally thought that the transfer of fluid into the ventricles involves active secretion by the epithelial cells as well as filtration. It is said that other fluids, the hormone or hormones of the posterior lobe of the pituitary, pass up the stalk and enter through the floor of the third ventricle. The fluid or fluids proceed in a sluggish fashion from ventricle to ventricle by the connecting foramina and a small part may pass in the central canal down the cord. The current is from the lateral ventricles to the third ventricle and thence into the fourth ventricle. Not so much

from the fourth ventricle anteriorly, because, when the communication between the third and the fourth is blocked, fluid accumulates in the third and lateral ventricles to such a degree that internal hydrocephalus results.

The principal escape of fluid from the ventricles into the subarachnoid space is through the roof of the fourth ventricle in which a median and two lateral foramina have been described, but it is still undecided whether actual openings exist or whether we have to do simply with areas of increased permeability. Since, therefore, the roof of the fourth ventricle is active through its chorioid plexus in the production of ventricular fluid, and through its foramina with the removal of fluid, it is obvious that fluid exchange is likely to be greater here than in other segments of the system. In its floor are situated automatic regulatory centers of the greatest importance.

The subarachnoid fluid also receives contributions from the living nerve cells. Weed (1923*a, b*) has shown that each nerve cell, or neurone, is enclosed in a perineuronal space which becomes continuous with pericapillary and perivascular spaces, opening ultimately into the subarachnoid space as represented in figure 207. These channels may serve, as the lymphatics in other tissues do, as a kind of overflow or absorptive system for the tissue spaces. They differ, however, from real lymphatics (which are absent in the brain) in a number of particulars. They are continuous in the brain substance with the tissue spaces, whereas lymphatics begin blindly, their contents being separated from those of the tissue spaces by a delicate endothelial wall. They are not lined with endothelium anywhere in their course. At the beginning they are simply clefts, but gradually are limited internally (around the vessel) by a thin investment of arachnoid mesothelium and externally (next the nervous tissue) by a similar layer acquired by the vessel in the course of development when it grew into the brain carrying the pia mater with it. There are no valves and the fluid, slowly making its way into the subarachnoid space by innumerable passages of this sort, is ordinarily free from cells. In certain pathological conditions these perivascular spaces may become loaded with lymphocytes which have wormed their way through the walls of the vessels and with activated microgliaocytes. The proportion of subarachnoid fluid arising in this way to that coming from the ventricles is not known, neither is its composition.

Evacuation from the subarachnoid space is now fairly well understood. There is some diffusion across the arachnoid membrane into the subdural space but particulate matter is held back. The principal avenue of escape is through the arachnoid villi into the venous sinuses. A villus is represented in figure 207. It projects into the sinus as an intestinal villus projects into the lumen of the gut. The sinuses are held open by their firm investment of dura and by the fact the bony wall of the skull is partly excavated to accommodate some of them. The penetrating end of each villus is made up internally of arachnoid mesothelial cells and externally of an envelope of vascular endothelial cells. Most investigators are of the opinion that the fluid passes out into the sinuses by filtration, that is to say, there is no evidence that any change occurs in its properties by secretion. The cellular barrier is not composed of epithelial cells. There has been much discussion of another means of exit along the nerve trunks which leave the brain case. A small amount of colored material has been observed to make its way out in this way with the fibers of the olfactory and optic nerves and to be gathered in by the local lymphatics, to be eventually dumped into the venous system. For details see Scholz and Ralston (1939).

## SUMMARY

The vital unit is a nerve cell in which irritability and conduction are developed to an extraordinary degree. It is long and thin as befits a conductile element and its structure is highly specialized. Ordinarily the impulse is received by several relatively short dendrites conducted to the cell body and discharged by a single long axone to the cell next in series. The cell body is the widest part contains the nucleus and is capable of regenerating new processes after injury. These vital units are grouped into larger units or reflex arcs, which give perception, conduction and end-effect. Stimuli resulting from changes whether they be external (somatic) or internal (visceral), activate receptors whose irritability is increased for certain kinds of stimuli and decreased for others. The impulses generated are conducted by sensory (afferent) cells to nervous centers or adjustors where they bridge over the synapse and activate motor (efferent) cells the axones of which pass away from the centers to muscles or glands (effectors). It is in the centers that integration takes place. The motor cells can be activated by impulses received from other receptors so that their efferent axones may constitute the *final common path* for the motor response in somewhat the same manner that the single axone is the final common path into which the impulses received by several dendrites are collected. The motor cells can also be inhibited by impulses travelling down from the brain. Neurologists alone are able to find their way in the bewildering maze of central connections. These have, however, been developed in a perfectly definite order in the course of evolution under the guidance afforded the head and the body by the *distance receptors* of the eye and ear but particularly of the nose. The pattern of arrangement of cells and fiber tracts is inherited and also certain special attributes. Training leads to the formation of certain nerve cell connections and associations and use stabilizes them. The mechanism is constructed for service during the entire life of the individual. Some memories must be lasting if he is to profit by experience. When parts are destroyed they are not replaced as in other systems but there is a margin of safety in the number of nerve cells. A few of them may die—especially those which do not occupy the key positions—without apparently interfering with the function of the whole. Energy is conserved wherever possible. Some cells like those in the respiratory center, must labor as long as we live. Somatic motor cells rest in sleep about one-third of the time though they may continue to discharge impulses that are less potent than usual. The receptors discriminate and inform us only of a limited series of changes in our external and internal environments. Otherwise the news received would lose point by its very volume and would monopolize our attention. To the same end we are spared the conscious direction of many visceral activities which are effected by the autonomic nervous system with the coöperation of the endocrines.

The central nervous system like the bone marrow which supplies the material for a slower but none the less essential means of integration and the pituitary the regulator of the endocrines is sheltered by rigid walls. It is moreover cushioned in fluid which is also made to act as lymph for there are no true lymphatics. The ventricular fluid is formed by filtration and secretion of the choroid plexuses. It enters the subarachnoid space mainly by passing through the roof of the fourth ventricle. There is in addition a slow seepage from the tissue spaces and along the perivascular channels into the subarachnoid space. Some subarachnoid fluid

may diffuse into the subdural space, which is itself a closed body cavity, though its width is very slight, and a little may leave *via* nerve bundles, especially the olfactory and optic, but the most effective avenue of discharge is by filtration through the arachnoid villi into the venous sinuses. The spaces traversed are of three kinds. (1) Derivatives of the original lumen of the neural tube (ventricles, interventricular foramina and central canal) lined with epithelium, called ependyma, which is not phagocytic but may be locally secretory (chorioid plexuses). (2) The perineuronal and other tissue spaces, which have no limiting membranes and the fluid of which is evidently in most intimate contact with nerve cells and fibers and almost stagnant. (3) The larger perivascular spaces, and the subarachnoid space, which are lined with mesenchyme, the cells of which are highly phagocytic given the appropriate stimuli.



## CHAPTER XX

### PRINCIPAL SENSE ORGANS

THE eye and the ear were evolved to serve our remote ancestors who were inhabitants of the sea. Today we look through thin films of salt water and become equilibrated by virtue of the presence of little masses of salt water deeply placed in our temporal bones. Rather than to elaborate new mechanisms Nature continues to supply these fluids so that the old mechanisms can remain in service with but slight modification—a good example both of conservatism and of the usefulness of water.

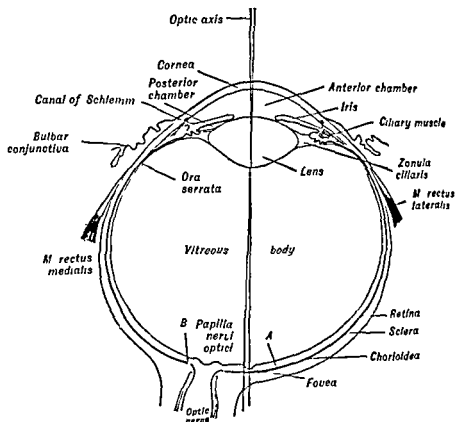


FIG. 208.—Diagram of a horizontal section through the right eye. A and B mark about location of sections illustrated by figures 213 and 215. (From Maximow-Bloom Textbook of Histology, W. B. Saunders Company.)

**Eye**—The eye is constructed like a camera for there is present an iris diaphragm, a lens and a photosensitive surface the retina. But it must receive rays of light through water and must serve the individual longer than any camera. Henshaw (1942) points out that the normal life span of the eye as a functioning organ exceeds that of the body as a whole. Only 25 per cent of persons over one hundred years of age are blind. He calculates that if we lived long enough the number of survivors with vision would not be reduced to zero at an age younger than one hundred and twenty or one hundred and thirty years. Certainly no other organ exhibits more interesting structural features. Consider these in order from without inward (Fig. 208).

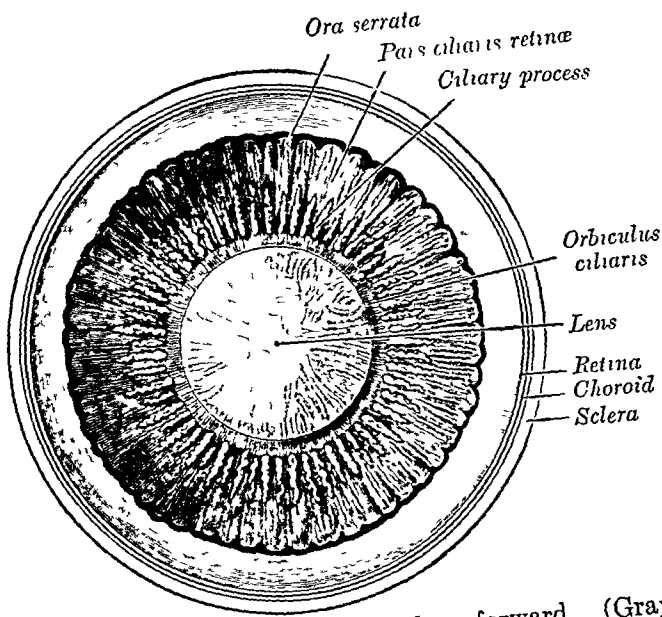


FIG. 209 —Interior of anterior half of bulb of eye looking forward (Gray's Anatomy).



FIG. 210 —Harderian gland of white female thirty-five years old who died of cerebral hemorrhage. Shows serous acini with cells loaded with secretion antecedents and spherical nuclei. Formalin fixation and H & E (Given by Dr T. E. Sanders.)

The lacrimal fluid that bathes the outer surface of the cornea and conjunctiva and lubricates the inner surfaces of the eyelids is of thin watery consistency. Its composition is probably not so simple as is usually thought. Sodium chloride is present in considerable amounts, but, since there is much evaporation the salt content of the fluid as secreted is obviously less. A peculiar stable substance known as lysozyme (Glycine solution + zymolysate) which brings about the solution of some bacteria has been identified. It is found also in egg white, urine, etc.

The *lacrimal gland* is divisible into two parts, superior and inferior. Both are of the same character and in the recent literature are called the *Harderian glands*. They are the principal source of the fluid. In a 'baby course' this gland is dispensed with in a few words, but the further one looks the more involved becomes the problem of its physiological role. In vitamin A and B<sub>2</sub> (riboflavin) deficiencies

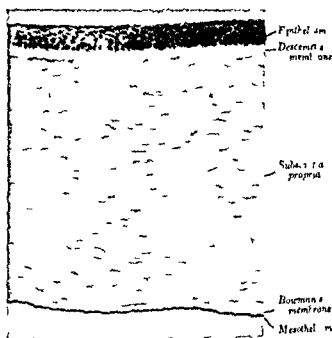


FIG. 211—Cornea of white male aged thirty-six years. Observe avascularity. Zerkow fluid H & E.  $\times 140$ . (Given by Dr. T. E. Sanders.)

vascularization of the cornea is correlated with disappearance from the gland of yellow material (Bessey and Wolbach 1939). Griffin (1942) quotes Wolbach as saying that when the vitamins are restored to the diet the vascularization regresses and the pigment reappears. The red fluorescence of the gland is probably caused by the excretion of porphyrin (Griffin 1942) and the intensity of the fluorescence seems to be in some way linked with the incidence of cancer in different strains of mice (Strong and Liggett 1941). Microscopically the lacrimal is a little like the parotids and like them may be involved in mumps. In both the dominant cells are of the serous type with many acid-stainable secretion precursors (Fig. 210) but in the lacrimal mucous acini and strongly acidophilic secretory ducts are absent.

The *cornea* is a strong, curved sheet of tissue forming the outer boundary of the anterior chamber. It is divisible into the layers shown in figure 211. The stratified

epithelium is really modified epidermis in which the tendency to pigmentation inherent in ectoderm is entirely suppressed in man, just how we do not know. There is slight pigmentation in certain mammals, even in one so closely related as the chimpanzee (Ida Mann, 1932). The time has not been accurately measured in man, but experimental injuries to the mammalian corneal epithelium heal in the remarkably short space of six hours (Arey and Covode, 1937). Beneath the epithelium is the conspicuous hyaline basement membrane of Descemet, followed by a thick substantia propria consisting almost wholly of collagenic fibers, fibroblasts, occasional nerve fibers and tissue fluid, but without blood vessels and lymphatics. The substantia propria is limited internally by the thin hyaline membrane of Bowman followed by a layer of mesothelium which constitutes the internal surface of the cornea.

Owing to its avascularity, exposure to the air and loss of heat by evaporation of water, the cornea is probably geared to work at a lower temperature than any other tissue in the body. Ida Mann (1932) cites an old report that its temperature is  $10^{\circ}\text{C}$  less than the general body temperature. Normally the cornea is kept clean by the lacrymal fluid spread by the winking reflex. Impulse to remove foreign bodies is given by the sensation of pain. When this is lacking, after cutting the fifth nerve proximal to the Gasserian ganglion in operations for relief of neuralgia, the pain fibers no longer function so that information of injury is lacking and infections are more frequent. When particles are not dislodged they may be covered with a smooth coating of mucus from glands of the eyelids. Invasion of the blood stream by bacteria is difficult because of the repeated washing of the surface with fluid containing some lysozyme, the barrier afforded by the closely fitted together epithelial cells and the long distance that the organisms must travel before they can enter the vessels encircling the cornea while subject to attack from leucocytes meeting them in the tissue fluid en route.

The aqueous humor in the *anterior chamber* is a tissue fluid of the second order. Interposed between it and tissue fluid of the first order is the ectodermal epithelium covering the ciliary process through which it diffuses as peritoneal fluid must pass through mesothelium. The direction of slow circulation is into the posterior chamber, thence into the anterior chamber by passage between the iris and lens, and out of the anterior chamber by the canals of Schlemm into the venous blood stream (Fig 208). This fluid is unique among body fluids thus far investigated for antibodies do not freely enter it from the blood plasma (Saphir, Appel and Strauss, 1941). It apparently does not exhibit certain species differences. Thus, Greene (1938) was able to grow human cancers in the aqueous humor of infrahuman species. Because of the transparency of the cornea the anterior chamber is a splendid site in which to observe the behavior of tissue transplants. Outstanding is the work of Markee (1940) on menstrual changes in transplanted uterine mucosa.

The *lens* is even more avascular than the cornea. By minimizing fluid exchange, this avascularity lends constancy to its optical properties. The lens is entirely ectodermal. Its anterior surface is covered by a thin, highly refractile optically homogeneous capsule. The internal substance is made up of greatly elongated epithelial cells, almost all of which have lost their nuclei and are styled lens fibers. The characteristic arrangement of the lens fibers is presented by Ida Mann. Only the anterior and lateral parts of the lens exhibit nucleated cells. A single layer of cuboidal cells, just within the capsule, is easily visible. This remains from the outer ectodermal margin of the lens vesicle. It was in the inner margin that the

ectodermal cells elongated so greatly that they filled up the cavity of the vesicle and transformed into the lens fibers making up the body of the adult lens. Certain reptiles in the Arizona desert instead of wearing sun glasses have their eyes impregnated with yellow pigment. See interesting account by Walls and Judd (1937) of the variety of intra-ocular color filters utilized by vertebrates.

Unlike a camera lens the optic lens changes its curvature in focusing. It is held in place by a suspensory ligament the *zonula ciliaris*, which extends between it and the firm sclera of the eye ball. Contraction of the ciliary muscle releases the tension on the ligament and allows the lens to round up and become more convex in near vision. At the same time the sphincter muscle of the iris contracts decreasing the aperture cutting down the light and giving better definition. In distant vision the ciliary muscle is at rest and the lens somewhat flattened by tension on the suspensory ligament.

The *vitreous body* lies between the lens and the retina. In the fresh state it is a clear material of a firm jelly-like consistency (*L. vitrum* glass). In fixed and stained preparations it looks fibrous.

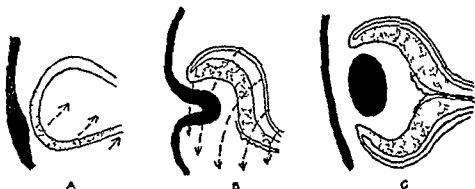


FIG. 212.—Diagram of invagination of the optic vesicle producing lens and double-layered retina. A Thickening of surface ectoderm (black) destined to form lens. The side of the vesicle that invaginates is stippled and the direction is indicated by the arrows. B The pushing in is almost complete so that the vesicle is like a double-walled goblet with the inner surface directed downward. The lips arch over in the direction shown. C The lips meet and fuse so that a double-walled goblet is now formed. The retina is thus made of two layers.

*Tissue transparency* marks the pathway of light up to the retina. Ida M. Strehlow stresses the following points: (1) The cornea and lens are the only transparent tissues in adult vertebrates. (2) All parts of young embryos are transparent except for yolk which is really not a tissue, opacity begins with the development of blood and the outer layer of the optic cup and gradually invades all the organs and tissues leaving only the cornea and lens clear. (3) In the evolution of animals many of the early forms are wholly transparent especially those that are aquatic. Opacity is again progressive until in humans transparency is limited to these components of the eye whose clarity is absolutely essential for vision. We may add another contrast that of surface and deep sea fishes. Though they may be quite closely related in a phylogenetic way many tissues which are opaque in the former are transparent in the latter. Attempts to explain this on the basis of absence of light, low temperature and great pressure in the depths of the ocean do not get us very far. For orientation a quantitative study of the actual degree of transparency of non-pigmented human tissues is desirable.

The *retina* is a part of the brain that has become photosensitive. In its development the outer surface of the optic vesicle becomes the inner surface of the retina—that to receive the light first—so that the branches of the central artery of the retina on the originally outer surface, and included when the two lips come together and fuse, are interposed between the retina and the vitreous body where they can readily be seen on ophthalmoscopic examination (Fig 212).

Since light bleaches visual purple, the image is outlined on the retina, where it has been photographed in the excised eyes of animals. Such photographs are called “optograms.” Criminologists have tried to discover in this way what murdered persons last saw, but the results are useless because the impression soon fades. The image is inverted, as must obviously be the case from the course of the light rays. The upright mental picture is developed through a readjustment in the optic centers in the brain.

Details as to the structure of the retina can always be found when needed in Polyak's (1941) book and in Detwiler's (1943) monograph on Vertebrate Photo-receptors. Only a few points need be remembered until after the examination. In ordinarily stained sections, taken at about the locality indicated by A in figure 208, search should be made for ten layers.

1 *Pigmented Epithelium*.—It will at once be noted that the external layer (remote from the vitreous body) consists of a single stratum of cuboidal pigmented cells (Fig 213). These supply a smooth surface to the chorioid and an uneven surface internally. The pigment is of the same brown color as the melanin of the chorioid, but evidence is lacking that it is the same chemically. It occurs in the form of discrete rod-shaped masses, distinct from the irregular globules of melanin, which are closely pressed together and tend to fuse. An interesting feature of the pigment, called fuscine (L. *fuscus*, dusky), is that it becomes heaped up in the protoplasmic processes of the cells that extend internally and leaves the nucleated external parts of the cells free and clear. In other words, it interposes itself between the nuclei and the rays of light in the same way that melanin behaves in the epidermis (p 372) despite the fact that it moves into a region of the cell which is embryologically proximal, in contrast to the distal position of the epidermal melanin. According to theory, the caps of melanin over the nuclei of the epidermal cells protect them by absorbing ultraviolet light, but this can hardly hold for the fuscine for it is unlikely that any ultraviolet will be able to traverse the aqueous humor, lens and vitreous.

2 *Rods and Cones*.—This stratum is a kind of meeting place of the pigmented epithelium, derived from the thinner outer wall of the optic vesicle, and of the outer nuclear layer, developed with all the rest from the inner, thicker invaginated wall (Fig 212). A fringe of pigmented processes extends into it from the former, and rods and cones from the latter. The rods and cones stain strongly with eosin. It is usually possible to distinguish them. The rods are longer and of even diameter, the cones shorter and more robust. Since both enter from the outer nuclear layer, the inner portion of the layer of rods and cones is more densely packed and is naturally more deeply stained. Nuclei are absent. No traces of visual purple can be made out in routine preparations, though this is where it occurs. It is along the line between rods and cones and pigmented epithelium that the retina is most likely to split in pathological processes and in the making of preparations. When detachment occurs the pigmented epithelium remains fixed to the chorioid.

3 *External Limiting Membrane* — This is a conspicuous thin sharply refractile acidophilic band formed by the terminal expansions of the neuroglial radial fibers. It really marks the external limit of the internal wall of the optic vesicle (Fig. 212) which the rods and cones project and interdigitate with the protoplasmic processes of the pigmented epithelium.

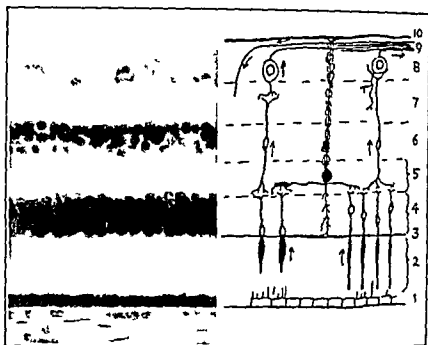


FIG. 213 — Section of retina made at about the point marked A in figure 212 with a diagrammatic representation of arrangement of cells. (Photomicrograph by James L. O'Leary of preparation of Harvey Lamb.)

4 *Outer Nuclear Layer* — Here are found closely wedged together the nuclei of rod and cone cells. Only traces of the cytoplasm can be made out. The rod and cone cells are the visual receptors and the first link in the chain leading to the brain.

5 *Outer Plexiform Layer* — Nuclei are again absent and as in layer number two the coloration is acidophilic in character. All one can see is a plexus of delicate fibers contributed by the rod and cone cells on the one hand and the cells of the inner nuclear layer on the other. It is the locus of contact between the first receptor cells and the second growth of conductile cells.

6 *Inner Nuclear Layer* — This is common with the outer nuclear layer is made up of thousands of spherical, deeply staining nuclei plus cytoplasm and fibers which require special methods for resolution. Here are cells of the second order.

7 *Inner Plexiform Layer* — This like the outer layer of the same name is fibrous, acidophilic, devoid of nuclei and the place of association of cells of the second order with the ganglion cells of the third order.

8 *Ganglion Cell Layer* — The cells are very much larger, globular elements with abundant cytoplasm in which distinct basophilic Nissl bodies can readily be seen. Their nuclei are larger and contain proportionally less basophilic chromatin. Solitary well formed nucleoli are often visible. They are cells of the third order.

and last order encountered in the retina. The large size may be related to the fact that the impulse received is transmitted not a few microns, but all the way along the optic nerve to the brain. The ganglion cells are less numerous than the cells of the first or second order. Usually they form a single layer. This is always true near the ora serrata, but elsewhere, except in the fovea and optic disc, they may be packed together five or more cells deep. Since, however, they are distinctly less numerous than the cells of the first and second order, it follows that most of them must receive impulses from more than one source.

9 *Nerve Fiber Layer* — It is in this layer that the axones of the ganglion cells stream toward the optic disc where they unite to form the afferent optic nerve. As one would expect, this layer increases in width as the fibers become confluent near the optic disc. In sections taken near the ora serrata it is barely noticeable. Some species have a few efferent fibers which pass down from the brain along the optic nerve and are distributed to the retina in this layer. Their function remains an intriguing mystery.

10 *Internal Limiting Membrane* — This membrane is less noticeable than the external one. But it is formed in the same manner, by expansion of the ends of the radial fibers. It is not unusual to find it slightly separated from the tissue beneath by the accumulation of a little fluid which may or may not be a post-mortem effect or an histological artefact.

The *visual receptors* have been subjected to most detailed investigation. The best account is that of Arey (1932). There are many theories as to how they function, which are considered in detail by the physiologists. Visual purple, rhodopsin (G *rhodom*, rose + *ops*, eye), occurs in the external segment of the rods, which stretches toward the pigmented epithelium, but is absent in the cones. The perception of brightness is assigned to the rods and of color to the cones. The latter are relatively most numerous in the fovea, where visual acuity is highest developed, and are said to be connected with the layer of ganglion cells in a more direct way. The common assumption that positional changes occur in the rods and cones and in the retinal pigment, fuscine, in man as well as in many lower forms (see Fig. 214) has evoked a strong protest from Arey. How the energy of light waves is transformed into an informative nervous impulse is a question that has led to many brilliantly conceived *theories of vision*. Of these the photochemical seems to be dominant. Zoth (1923) hypothecates a primary energy transformation aided by fuscine in the pigmented epithelium and a secondary transformation in the outer parts of the rods and cones by which these elements are stimulated and start the nervous impulse on its path to the brain.

The retina has a *blood supply* all its own. It is nourished by the central artery which enters with the optic nerve and is drained by the central vein leaving by the same route. The central artery of the retina is the best example of an end-artery. Because there is no collateral blood supply, when it is plugged the entire retina must die through loss of blood. Its branches spread over the retina in all directions. Scarcely a section can be studied without revealing one of them. They are evidently interposed between the visual receptors and the source of illumination. It is for this reason, when looking at the sky, with eyes adjusted for distant vision, we often see an irregular dark system of branching structures which disappear if we attempt to study them more closely. Owing also to the superficial position of the blood vessels, the ophthalmologist is able to look directly into the eye and study their changes from year to year. Until a method was



developed for the examination of capillaries in the skin the eye was the only location where this could be done. The point of entrance of optic nerve and blood vessels is not covered by retina so that it constitutes the 'blind spot' (Fig. 215). The

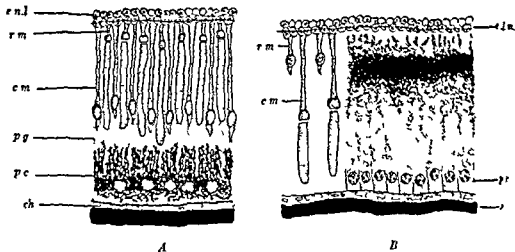


FIG. 214 — Retinal elements in *A* dim and *B* bright light vision in the fish *Ictalurus nebulosus*. *A* The pigment is withdrawn toward the choroid thereby allowing maximal utilization of dim light. The visual elements assume similarly advantageous positions: the relatively insensitive cones are extended out of the way and the functioning rods are drawn down near the image plane, *ch* choroid, *pc*, pigmented epithelial cells, *pg* photoreceptor granules, *cm* cone myoid, *rm* rod myoid, *enl* external nuclear layer.  $\times 450$ . *B* The pigment moves forward toward the external limiting membrane thereby masking and isolating the visual rods and cones, at the left of the figure the mutually advantageous positions of the visual elements are indicated: the sensitive rods are elongated and so are protected and out of use; the cones are shortened to the level of the image plane, *sc*, sclera, *pc* pigmented epithelial cell, *elm* external limiting membrane, *cm* cone myoid, *rm* rod myoid.  $\times 450$  (Redrawn and modified from Arey Special Cytology 1st Ed. Hoeber Inc.)

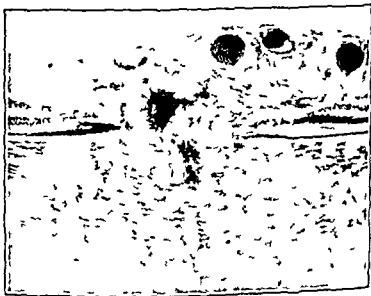


FIG. 215 — Photomicrograph of entrance of optic nerve into the retina at about the point marked *B* in figure 208. (Photomicrograph by James L. O'Leary of preparation of Harvey Lamb.)

retina, in common with the brain of which it is really a part, is devoid of *lymphatics*. Its cells do not normally multiply. They are fixed postmitotics, like nerve cells, gifted with long life.

**Ear.**—Structurally the ear consists of an apparatus for reception of sound waves (external ear), transmission (middle ear), tone analysis and equilibration (inner ear), but all the actual receptors function in fluid. The plan of architecture can never be appreciated without some knowledge of development. A readable and concise account is to be found in Bremer's *Histology*.

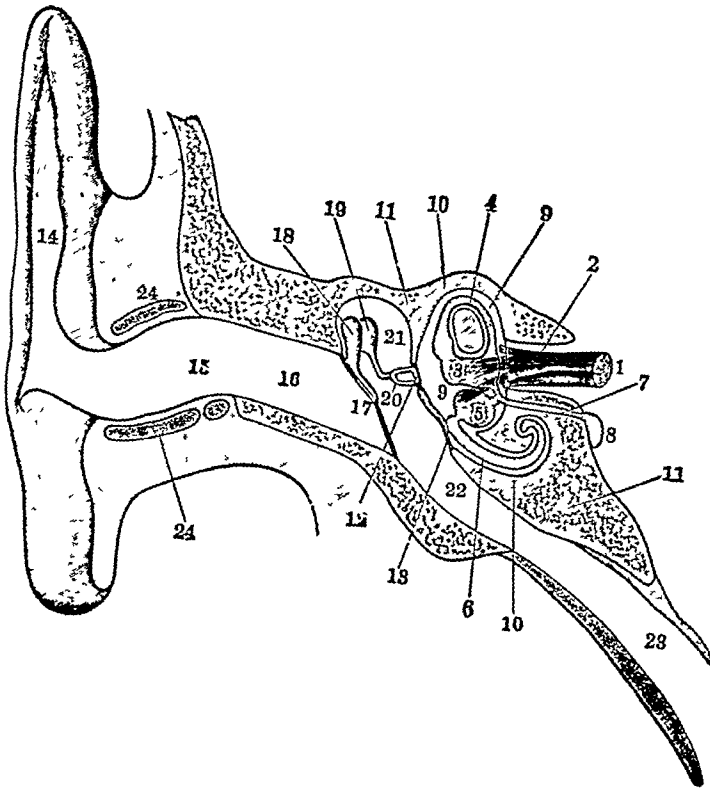


FIG 216 —Diagram of ear 1, Auditory nerve, 2, internal auditory meatus, 3, utricle; 4, posterior semicircular canal, 5, sacculus, 6, ductus cochlearis, 7, ductus endolymphaticus. 8, saccus endolymphaticus, 9, vestibule, 10, wall of osseous labyrinth, 11, temporal bone, 12, vestibular fenestra (with stapes applied), 13, cochlear fenestra, 14, auricula, 15, 16, external auditory meatus, 17, tympanum, 18, malleus, 19, incus, 20, stapes, 21, 22, middle ear, 23, Eustachian tube, 24, cartilage (Starling, after Schafer, J and A Churchill Company, Ltd)

One important event is the coming together of the first branchial cleft, forming the external ear, and of the first pharyngeal pouch, giving rise to the cavity of the middle ear and of the Eustachian tube connecting it with the pharynx. The line of contact is the tympanum, or drum membrane (Fig 216, 17). The surface of the external ear is epidermis of ectodermal origin, while that of the middle ear is mucous membrane of endodermal derivation. There is always a danger of forcing infective material up into the middle ear when pressure in the pharynx is raised by blowing the nose. When a perforation exists in the tympanum smoke can obviously be exhaled through the ear if the Eustachian tube is open.

The *mastoid antrum* opens into the middle ear posteriorly and is connected with many irregular mastoid air cells. These structures are not cells in the histo-

logical sense but spaces produced in the temporal bone by the process of pneumatization. Their development and arrangement are described by Smith (1932) and Meltzer (1934). With the slow resorption of bone in the mastoid process at birth mesenchyme enters from the mastoid antrum followed by epithelium and the spaces excavated are ventilated. Some bony partitions persist so that after

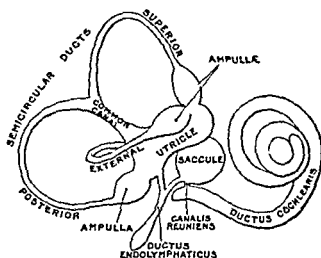


FIG. 217 —The membranous labyrinth. Enlarged. (Gray's Anatomy.)

pneumatization the inner part of the mastoid process is like a bony epithelium-lined sponge. Smith states that the reason why excavation is not complete as in the smooth-walled maxillary sinus is the relative absence of bone marrow in the latter. The epithelium is of the middle ear type but flatter and more firmly bound to the periosteum. The medial bony wall is much thinner than the lateral

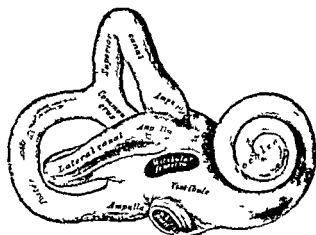


FIG. 218. Right osseous labyrinth. Lateral view. (Gray's Anatomy.)

one and the venous drainage is into the lateral sinus and dural veins within the skull cavity. The danger from infections is well known. Protection is given to the Eustachian tubes, middle ears, mastoid and air cells by the collapsible parts of the Eustachian tubes, the cleansing and protective serous and mucous secretions and the action of the cilia in sweeping down toward the pharynx as they dis-

the nasal passages For the anatomy of the tubes see Wolff (1934*b*), and physiology of middle ear see Lierle and Potter (1941)

Another event is the invagination of surface ectoderm owing to some organizing stimulus from the hind brain and head mesoderm (Needham, 1942). When this is pinched off it constitutes the otic vesicle In a truly remarkable fashion the vesicle grows inward and becomes transformed into the membranous labyrinth (Fig 217) In so doing it extends rather flat projections into 3 planes of space, the adjacent walls fuse in their middle portions and become absorbed so that the rims of the projections remain as the superior, posterior and lateral semicircular canals based on a portion of the body of the vesicle, termed the utricle The remainder is moulded until finally the utricle is only connected by a narrow tube with another expanded portion, the saccule From this tube, the ductus endolymphaticus (Fig 216, 7)—a vestige of the original stalk connecting the otic vesicle with the surface—reaches through the temporal bone and ends in the dura in the blind saccus endolymphaticus Because the saccus is in the dura and not beneath it, as insisted long ago by Retzius and proved by Wolff (1934*a*), it is seldom seen from the surface and never after the dura is stripped from the bone A small canal, the ductus reuniens, joins the saccule to the coiled ductus cochlearis The membranous labyrinth contains endolymph and its epithelial wall, of ectodermal origin, is supported by connective tissue

The word "endolymph" is unfortunate The fluid in question is not internal lymph It is a tissue fluid of the second order comparable with the aqueous humor of the eye, for interposed between it and the blood stream is not only vascular endothelium but also a layer of epithelium Guild (1927) has investigated the circulation of endolymph by introducing a solution of potassium ferrocyanide and iron ammonium citrate into the cochlear duct of living guinea-pigs, killing them at intervals up to forty-four hours and fixing the excised tissue in an acid mixture which precipitates Prussian blue at the place where the solution has moved to Since the Prussian blue accumulated in the endolymphatic duct, he concluded that this was the principal site of overflow of fluid In support of this contention he cited the accumulation of desquamated cells in the duct, presumably washed thither by the current and left behind when the fluid passed out Where the fluid goes to is another question It may get into the blood stream or cerebrospinal fluid.

The third event, occurring more or less synchronously, is the orderly accumulation of fluid (perilymph) in the embryonic connective tissue about the developing membranous labyrinth By contrast this is tissue fluid of the first order since it is not walled in by epithelium In this perilymphatic space the membranous labyrinth is adherent to the surrounding periosteum or is anchored to it by connective tissue strands Since the first layers of bone are especially hard (Fig 216, 10) the entire mass can be separated from the surrounding temporal bone and is designated the osseous labyrinth (Fig 218) Within this firm casing are then two fluids, perilymph and endolymph, the relations of which are the keys to the mechanism Three features are to be noted

(1) The ossicles in the middle ear conduct vibrations of the tympanum to the vestibular fenestra in the wall of the osseous labyrinth where they impinge upon the perilymph first in the vestibule and thence throughout its extent, the stapes acting as a kind of plunger

(2) The vibrations in the perilymph investing, or close to, the portions of the membranous labyrinth concerned with equilibration have no stimulating influence

on receptors of the vestibular division of the acoustic nerve. Totally deaf persons can equilibrate.

(3) The cochlea is however constructed in such a way that the spiral duct, cochlearis of the membranous labyrinth is sandwiched between the columns of perilymph ascending from the vestibule in the scala vestibuli and that descending in the scala tympani (Figs. 219, 216, 19). The latter ends blindly against the membrane of the fenestra cochleæ, but the old view that this is a necessary shock absorbing mechanism by which the membrane bulges out into the middle ear where pressure is exerted on the perilymph in the inner has not been substantiated. Hughson and Crowe (1931) have found that fixation of the membrane of the fenestra cochleæ by cement does not inhibit sound transmission, indeed, pressure exerted on the membrane from the middle ear improved auditory acuity. They look upon this fenestra as a kind of safety valve dampening the pressure and preventing overstimulation. Perhaps it may also have a directional function. The actual

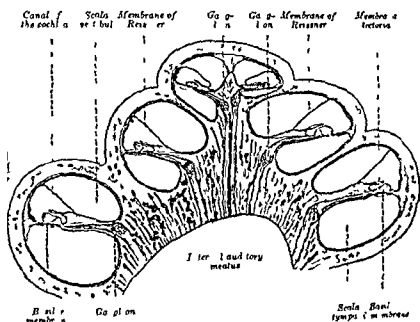


FIG. 219—Vertical section through the middle of the cochlea. (Shepey-Schafer, *Histology*, Longmans, Green & Co.)

placing of this membrane between the perilymph at the end of the scala tympani and the middle ear instead of, say, between the perilymph of the vestibule and the middle ear near the stapes may serve by its elastic quality to absorb pressure waves to conduct these waves through the scalae and thus in some way to concentrate them on the receptors of the cochlear division of the acoustic nerve in the duct cochlearis.

The receptors for both divisions are hair cells. The vestibular ones are arranged in 5 clumps—in 3 cristæ within slight dilations or ampullæ where the circular canals join the utricle and in 2 maculæ (1 spot) within the utricle and sacculus respectively. The pattern is uniform. The hair-like processes extend into material of gelatinous consistency covering each crista and each macula. It is thought that when the head moves the endolymph lags a little behind by inertia and that slight traction on the material in touch with the

hair cells stimulates them. The influence of the columns of fluid in the semicircular canals on the cristæ is not difficult to understand but how the maculæ function is obscure (Tait, 1932). The mechanism, in its essential features, was adopted by Nature in the very remote past. When iron filings are substituted for otoliths in the crayfish the orientation of the animal can be controlled by a magnet. To better understand equilibration has now become a war problem (McNally and Stuart, 1942).

The cochlear receptors are much more complicated. The hair cells extend in bands through the length of the ductus cochlearis and rest on a specially constructed fibrous basilar membrane (Fig 220). They are in touch with, or close to, a well placed tectorial membrane which, like the basilar membrane, decreases progressively in size as the end of the ductus is approached. Space does not permit a discussion of theories of audition: but something vibrates, probably in a different way at different levels in the cochlea, and this, picked up by the hair cells, is interpreted in the brain as sound.

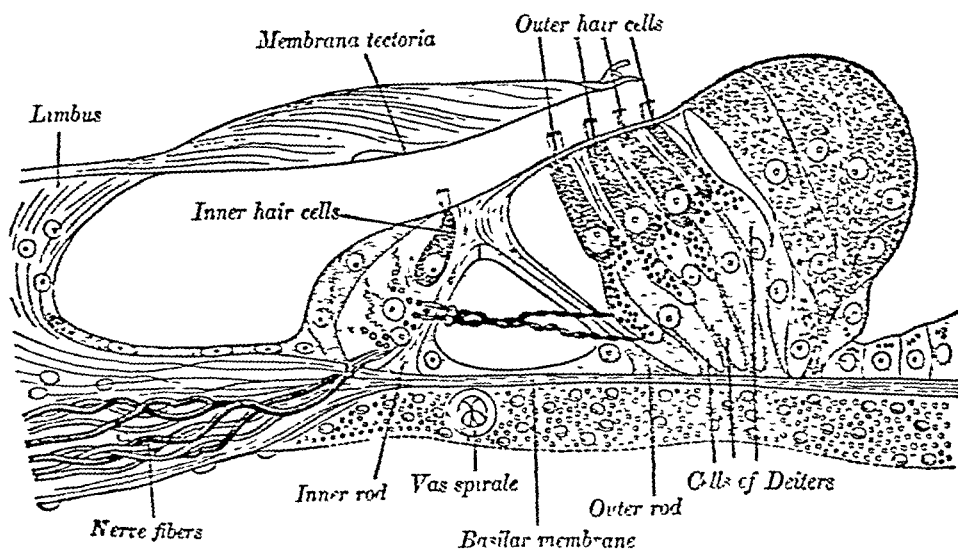


FIG 220.—Section through the organ of Corti. (G. Retzius.)

It is clear that special fluids are developed for the sake of hearing and equilibrating as well as for seeing. Ageing of both is considered by Guild (1942). There is first a decrease in perception of high pitched sounds. In England the onset of old age was graphically described as the inability to hear bats squeak. Guild states that the absence of reports on failure of the vestibular apparatus may only mean that the methods of testing are too crude to reveal small differences. Perhaps this ancient apparatus has a long life span like the eye. Useful reviews of current literature on the anatomy and physiology of the ear are published regularly in the *Arch Otolaryngol.* (See Richardson and Holmes, 1942)

### SUMMARY

The eye and ear are described as distance receptors because they inform the body of environmental changes which may originate far away, but they are also contact receptors for the energy of light and of sound must be brought into contact with living cells before it can be perceived. But equilibration by the vestibular division of the auditory nerve is on a different basis. The information given is

proprioceptive, relating like that furnished by the muscle spindles to the position of the body in space. All three hark back to the time when our ancestors were aquatic. To this day we see, hear and equilibrate through persistent local and specialized watery environments. No student can be expected to long remember the ten layers of the retina but he will recall certain similarities between the eye and a photographic camera—the iris diaphragm, condensing lens and photosensitive film or layer behind—also the utilization of transparent and avascular materials. The ear is constructed on a different plan. Sound waves are gathered in by the external ear. These are transmitted by the ossicles through an air-filled space (middle ear) borrowed from the first pharyngeal pouch, and impinge upon the fluid of the inner ear, vibrations of which in some way stimulate the cochlear division of the eighth nerve. Equilibration is also served by stimulation of terminal of the vestibular division of the same nerve, likewise in a fluid environment buried deeply in the hard petrous portion of the temporal bone but in a physiological sense part of the original outside watery surroundings. Protection of the surface of the eye is afforded by the thin aqueous secretion of the lacrimal glands and by the action of the eyelids like window wipers on an automobile designed to throw away dust and dirt but to leave behind a thin and even film of water. The middle ear is protected against invasion by pathogenic microorganisms by the closure of the Eustachian tube, except during swallowing, and by a clearance mechanism of secretions and cilia comparable to that in the respiratory tract. Replacement of the sensory cells of the eye and ear is conspicuous by its absence. Normally these are never seen in mitosis. But the surface epithelial cells of the cornea shield the optic lens and of the middle ear are replaced when worn out and desquamated by multiplication of existing cells, while a few of the unspecialized cells lining the membranous labyrinth are also cast into the endolymph and their place taken by others as in epithelia throughout the body.

## CHAPTER XVI

### CONNECTIVE SYSTEM

THE blood integrates by the transport of material and the nervous system by the sending of messages. It is natural to think of the connective system as integrating by the binding of the tissues and organs into a unit. This degree of mechanical association of parts varies from that of the loose connective tissue beneath the skin and between and within the organs, the cushioning effect of fatty tissue, the elasticity conferred on blood vessels by elastic fibers, the yielding support of cartilages to the rigid framework of bone, and the firm, durable connections of tendons and ligaments. Loose connective tissue integrates in another manner by affording easily followed pathways through which the nerves, blood vessels and lymphatics gain access to the organs. Integration by connection is only part of the story. The system provides also for separation. Ball and socket joints, like that of the shoulder, afford examples of both connection and separation. The bones are connected by a capsule, but the moving surfaces are separated by a thin fluid lubricant and coated with special friction-reducing tissue. Where viscera have to move against viscera each is limited by an investment of slippery mesothelium and between the two more lubricant is interposed (cf. the peritoneal and pericardial fluids). In other places separation is provided by connective tissue derivatives where movement is not involved, as by the firm dura mater and the delicate pia arachnoid. Organs throughout the body are supplied with capsules which separate the cells within them from the rest. This separation and segregation of activities is essential. If the spleen, suprarenal and kidney were all merged together they could not function. The cells in each, shielded by connective tissue, have developed certain capabilities and the kind of local environment required for the performance of special duties. Even in the cell itself it is the separation and localization of reactions which makes life possible. The separation of nuclear from cytoplasmic material by the development of a nuclear membrane is fundamental. Within both nucleus and cytoplasm there is likewise marked localization on the surface of things visible and invisible. In other words, separation of the body into special compartments by connective tissue partitions, and of the cell into distinct areas by other membranes, and by the presence of substances so different physically that they develop interfaces, is every bit as necessary for vital activities as the spatial organization of a manufacturing plant. If the barriers were broken down and everything mixed together in the body, the cell, or the factory, nothing useful could be done.

**Mesenchyme.**—All connective tissues, as well as blood, lymph, R. E. cells and some smooth muscle, are derived from *mesenchyme* (G. *mesos*, middle + *enchyma*, infusion), which is a kind of thin infusion or mixture of cells originating from the middle germ layer. The cells are irregular bodies which may possess quite long processes. The older anatomists were convinced that these were united by numerous anastomoses to form a true syncytium (G. *syn.* with + *kytos*, a hollow, a cell), but this is probably not the case (Lewis, 1922). Mesenchymal cells are



typically suspended in a relatively large volume of tissue fluid. They do not form compact masses like epithelial cells. With tissue fluid they fill in the spaces between the developing organs of the embryo in the same fashion that loose connective tissue is utilized as the chief packing material in the adult.

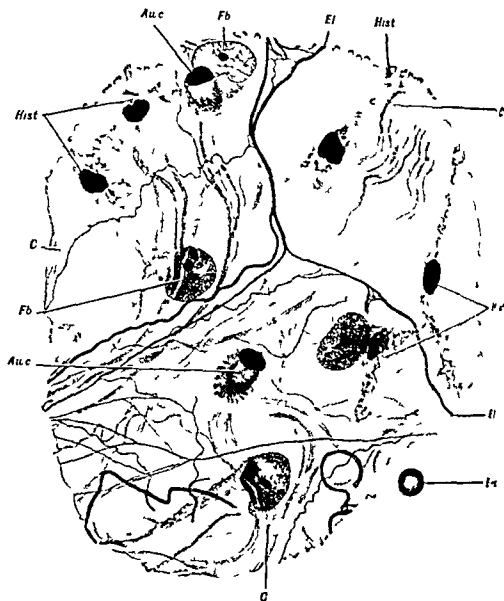


FIG. 221 — Section through slightly edematous subcutaneous loose irregular areolar connective tissue from the thigh. *C* Collagenic fibers. *El* elastic fibers. *Hist* histiocytes. *Fb* fibroblast. *Auc* amoeboid wandering cells. *Erc* erythrocyte for size comparison. *I* interstitial space. Hematoxylin stain.  $\times 950$ . (Maximow Bloom Textbook of Histology W. B. Saunders Company.)

**Loose Connective Tissue** — This is a direct differentiation from mesenchyme and one of the most important constituents of the body. It is also called *areolar* because it presents many little areas (*areola* = dim. of area) in which no structure is ordinarily seen. To Sylvia H. Bensley (1934) belongs credit for finally demonstrating after years of controversy that the tissue fluid in which the cells and fibers are imbedded is not always simply a thin watery fluid but a material that

can be rendered microscopically visible. In young, undifferentiated connective tissue this material is continuous, of viscid consistency (preventing the free movement of fine particles) and mucoid in nature. It stains metachromatically. In adult subcutaneous tissue, on the other hand, it is less abundant being concentrated about the fibers so that relatively large tissue spaces are free from it and contain only fluid which is not jelly-like. This intercellular ground substance undergoes definite changes in normal differentiation, rhythmic phenomena in the uterine mucosa, fibrosis and other alterations.

In spreads of subcutaneous tissue mounted in salt solution the tissue fluid is naturally invisible, but the following structural components are recognizable:



FIG 222 —Collagenic fibrils from fresh tendon of rat's tail teased in water. Viewed with electronmicroscope  $\times 26,400$  (Schmitt, *et al*, courtesy of J. Cell and Comp. Physiol.)

*Collagenic fibers* are conspicuous objects of low-refractive index which show a longitudinal striation owing to the fact that they are made up of many small fibrils plastered together. The designation "collagenic" is appropriate because on boiling they yield collagen which, in the hydrated condition, is a gelatin. The collagenic fibers are by far the thickest ones in the spreads and pursue a kind of wavy course. When a dilute acid is added they swell, but not evenly throughout their length. In some places they are very characteristically constricted for some still obscure reason. Seen in ordinary H and E preparations collagenic fibers frequently stain more intensely than the smooth muscle as is illustrated in figure 40.

It may be desirable to reduce the length of this book but not at the expense of collagenic fibers. Without them the body would quickly fall apart. Much depends

on their maintenance in good condition and their prompt development in wound healing so that they are now beginning to receive the close attention that they deserve. Healing of tendons composed chiefly of these fibers is a problem of great interest (Masson and Allen 1941).

The electron microscope does more than 'make chromosomes look like snakes,' it shows the quite unsuspected fact that collagenic fibrils that make up the fibers are cross striated (Fig. 222) as reported by Scott and Anderson (1941) and by Schmitt Hall and Jakus (1942). The latter find this banding to be present in all the collagenic fibrils examined that is from 4 species of mammals (including man) 1 amphibian and 1 mollusk so that it is a fundamental structural property. They also call attention to the great extensibility of individual fibrils and state that the non-extensibility of fibers depends on lateral binding of the component fibrils. It is likely that this kind of study at very high magnification can be linked with x-ray analyses of collagen with the result that both the character and the grouping of the molecules will become clear.

Collagenic fibers are susceptible to experimentally imposed changes in hydration concentration and to increase in temperature. Baumberger and his associates (1942) have discovered that the grip of collagenic fibers of the dermis on the overlying epidermis can be loosened by a swing of reaction to either the acid or alkaline side as well as by increase of temperature to 50° C for two minutes. The change from gel to sol state is reversible for after the influence is removed the epidermis becomes again bound to the dermis. There is as yet no reason to think that the fibers are influenced by the less marked changes in pH and temperature to which they are subjected *in vivo*. But since collagenic fibers serve in so many different environments singly in loose connective tissue in bands in the adventitia of blood vessels (p. 59) as reinforcing strands in the ground substance of bone and cartilage in tendons to mention only three it would seem unlikely that chemical surroundings and physical strain would act equally in all cases. By employing the very useful transparent chambers in rabbits' ears, so often referred to as Stearns' (1940) has worked out the rate of formation of fibers and demonstrated that presence of fibroblasts is essential.

Battell has in several contributions (see Battell and Mason 1934) reported experiments which indicate that the collagenic fibrils are a transformation product of fibrin and that they develop extracellularly in the tissue fluid. An important advance is that of Wolbach (1933) who availed himself of an observation which he made with Howe (Wolbach and Howe 1926) that the deposition of intercellular

#### LEGEND FOR FIG. 223

FIG. 223 - Figures showing the controlled formation of collagen and reticulum in blood clots of guinea pigs rendered scorbutic by deprivation of vitamin C and later recovered after the deposition of intercellular substances owing to the feeding of orange juice. All specimens were fixed in Zenker's fluid and colored with Mallory's connective tissue stain except those of 6 and 8 which were treated by Foot's modification of the Bielschowsky-Marschall method before staining. All  $\times 1000$  except figure 8  $\times 430$ . 4 Collagen (in blue) deposited about an isolated cell. Recovery period seventy-two hours. 5 Collagenic advanced fibrin strands in red. 6 Fine argyrophilic fibrils (green black) appear. Argyrophilic fibrils no longer noticeable. A later stage. Repair ninety-six hours. 8 Argyrophilic fibrils in endochondral bone formation. 9 Endosteal surface of rib in alkydite section. Note fibroblasts and fibroglia fibrils (in red). 10 Similar cells active at osteoid lasts in 1 hour recovery period. (Wolbach courtesy of American Journal of Pathology)

4

5

6



9



8



10

FIG 223

materials cannot take place in animals deprived of vitamin C to the development of absolute scorbutus although proliferative reparative processes are active. Cell formation begins however twenty-four hours after the feeding of orange juice. Consequently he was able experimentally to control, time and rate the changes that took place in the repair of blood clots in guinea pigs. The fibroblasts first appeared as a diffuse deposit about them which he interpreted as a secretion (Fig. 223, 4). The second stage was the differentiation of delicate fibrils with collagen (Fig. 223, 5) which began to exhibit an affinity for silver in order not to become argyrophilic (Fig. 223, 6) owing, he thinks, to their extreme delicacy. The fibrils were disposed about the cell bodies as centers. But in cell clots the fibrils coursed irregularly between the cells and it was impossible to associate a group of fibrils with a definite cell. More mature collagen lost its argyrophilic property (Fig. 223, 7). He observed the same sequence in the resumption of bone formation following the orange juice treatment (Fig. 223, 8). The endosteal surface of a guinea pig's rib in complete scorbutus is illustrated in figure 224. Cells indistinguishable from fibroblasts "showing many fibroblastic fibrils penetrate into the bony substance and in recovery become osteoblasts. Wollbrich's conclusions were that (1) In repair by reorganization collagen is not derived from fibrin or other preformed substances. (2) Collagen and reticulum are the same material in different physical states. (3) Collagen is secreted by the fibroblasts and its distribution is determined by the shape of the cell and position of its fibroblastic fibrils.

To explain exactly what the red stained fibroglia fibrils are in contrast to the light blue collagenic ones, is difficult. Mallory who introduced the term was of the opinion that fibroblasts give rise to two kinds of fibrils: fibroglia fibrils which are related to them in the same way that neuroglia fibrils are to neuroglia cells and collagenic fibrils which are extracellular and disposed between them. According to Penfield the glia fibrils are probably within the processes of the astrocyte (neuroglia cell) just as Wollbrich shows fibroglia in the fibroblasts. But evidence is unconvincing of the existence of any fibrils in still living fibroblasts last removed from the body or in fibroblasts in tissue cultures. Mallory states that the fibroglia fibrils are best studied in actively growing connective tissue as for instance in the stroma of carcinoma in chronic salpingitis and other situations. In such cells these structures colored so vividly in Wollbrich's figures are a natural part of some sort in the living cell or cell membrane in somewhat the same fashion that myofibrils and neurofibrils have in the cytoplasm. In figure 225 it can be noticed that the fibroglia fibrils often limit the cytoplasm and are more conspicuous in the cell processes which are more subject to strain during growth and differentiation and to shortening with the contraction due to fixation. As to the evident differences however one must be stressed that we concern ourselves with physical conditions responsible for fibroglia fibril development as noted especially in conditions like those mentioned by Mallory and less so in other cases so that sometimes they appear and sometimes they do not whereas after appropriate technical methods the myofibrils and neurofibrils always appear.

In the livers of rats Lowry and Hastings (1912) observed an increase in collagen from 9.7 gm. collagen per kg. at thirty days to 13.2 at one hundred days which gives point to the current idea of fibrosis with age but the increase may be relative (not absolute) occasioned partly by decrease in muscle and fat.

components. That there is some alteration in quality with advancing age is clear from the report by Evans *et al* (1943) that the retraction, or shrinkage, of excised dermis is less in old than in young people. How long collagenic fibers retain the physical properties on which their usefulness depends is a mystery. There are no data on the rate of turn over of collagen. But Pullinger and Pirie (1942) have discovered that implantation of collagen results in chronic inflammation and suggest that the break down of collagen may bring about the chronic inflammatory lesions so frequently encountered in elderly persons. They refer to Bergmann's earlier observation that, *in vitro*, digestion of collagenic fibers occurs only at the cut ends. Perhaps the breaking of fibers by trauma facilitates removal.

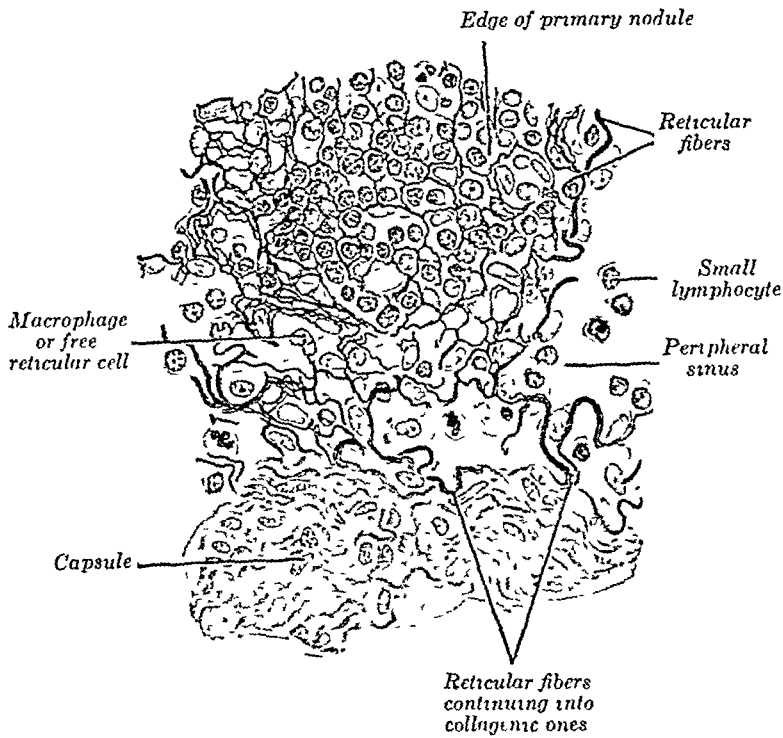


FIG 224 —Mesenteric lymph node from an executed man. Bielschowsky stain.  $\times 500$  (Maximow-Bloom, Textbook of Histology, W B Saunders Company)

*Elastic fibers* are entirely different. They are much less numerous and appear as delicate, homogeneous threads of high refractive index which branch repeatedly, forming a network. In other localities, where many of them are closely applied together, as in elastic membranes, the vocal cords and ligamenta flava, it can be seen that they are of a pale yellow color. Consequently they are sometimes referred to as *yellow fibers*, and the collagenic fibers, which have no color, as *white fibers*.

Chemically and physically these fibers are very different from collagenic ones. No particular vitamin appears to be required for their development. But there is a similar hiatus in our knowledge concerning them, particularly in respect to turn over of material, the possibility of inheritance of good and defective material and the influence of local conditions on ageing.

*Reticular fibers* are less readily seen in fresh unstained tissues. As the name suggests, they are not long filaments but form a reticulum which is close-meshed. Subcutaneous tissue is not a good place to find them. They occur most abundantly in epithelial organs where they supply a yielding support for the cells. They are

also an important component of lymph glands and blood forming organs. If the other elements are removed by tryptic digestion it is difficult to distinguish them. They may be revealed in permanent preparations by silver impregnation. The relation of reticular fibers to the other two has been much discussed.

### PROPERTIES OF FIBERS

	Collagenic	Elastic	Peticular
Terminology	Collagenic because they yield collagen on boiling Termed 'white' owing to lack of color	Elastic because made up of elastic colloid elastin Termed yellow owing to their distinctive color	Peticular because exposed in thin networks (L. reticularis a fine net) argyrophilic owing to affinity for silver
Occurrence	Widest distributed, concentrated in tendons	Blood vessels and distensible viscera vocal cords, ligamenta flava etc	Framework of lymph glands, bone marrow endocrines and other epithelial organs
Morphology	Broad sometimes wavy made up of many fibrils bound together by cement substance and limiting membrane Do not branch	Fine thin threads not fibrillar branch and anastomose freely	Networks thinner than collagenic
Physico-chemical properties	1 Refractive index, low soft flexible 2 General resistance to chemical change fair 3 Weak acids and alkalis swell and dissolve 4 Acid pepsin easily digested 5 Alkaline trypsin resistant 6 Hydrated collagen is gelatin	Index high elastic Perhaps most resistant material in body Resistant Slowly digested Slowly digested Contains 50 per cent glycine and leucine (Wells, 1933)	Index intermediate yielding More like collagenic Not so resistant ? Resistant Unknown
Diapictronal properties	1 H and E stains pink 2 Mallory blue 3 Orcein and resorcin fuchsin not stained 4 Various silver techniques, not usually blackened	Usually not colored Pink or yellow Purple-black Not usually blackened	But faintly pink Blue Not stained Specifically blackened
Formation	1 From fibrin? (Baitsell 1933) 2 In intercellular substance under influence of fibroblasts 3 Independent	From unknown substance Same Independent	Possibly from fibrin Same the fibrillar substance called reticular cells May be anatomically continuous with collagenic
Function	Principal binding material great tensile strength	Affords elasticity in arteries sclerosis exhibits phenomena of ageing like all colloids (Wells, 1933)	Supplies a yielding mass for cells, holding them in place

hold that they are fundamentally different Corner (1920) and others think that they are formed by vascular endothelium The most comprehensive account of all the connective tissues has been given by Maximow (1927), who emphasizes a relationship to collagenic fibers and shows instances of anatomical continuity between the two (Fig 224). Indeed, reticular fibers are frequently styled "pre-collagenic" Wolfe *et al* (1942) have described a gradual transformation of reticulum into collagen in the process of ageing of connective tissue in the female genital tract of rats

*Fibroblasts* (L *fibra*, fiber + G. *blastos*, germ) are descendants of the primitive mesenchymal cells from which it is often difficult or impossible to distinguish them It is, however, a simple matter to identify them without the addition of any stain in spreads of subcutaneous tissue. They occur by preference close to large collagenic fibers Their nuclei are oval with a tendency to lateral indentation and are much the largest ordinarily present The nuclei look empty but close study usually discloses one centrally placed nucleolus, seldom two or more The cytoplasm is usually rather free of granules and extends out into thin, rapidly tapering processes, the limits of which are somewhat indistinct In H and E sections fibroblasts are also easily identified by their elongated nuclei and by their position in association with collagenic fibers The only other nuclei with which they could be confused are those of endothelial cells marked by their relation to vascular or lymphatic lumina.

Carrel and Ebeling (1926) have advanced our knowledge by studying the food requirements of fibroblasts in pure line tissue cultures They say: "It became obvious that fibroblasts must be in a resting condition within the adult animal on account of their inherent property of requiring for multiplication substances which are not present in lymph or blood serum It is also evident that they are capable of assuming their embryonic activity, even in extreme old age, during the process of wound healing, or of organ sclerosis, because they have the power of feeding on substances set free by leucocytes or by epithelial cells, as happens when adult connective tissue is cultivated in embryonic juice" Presumably they mean by "resting" that the fibroblasts are simply playing their part in the maintenance of the tissue fluid and fibers about them for real inactivity means death The authors find that the increase in rate of growth caused by the addition of embryonic juice to the culture medium is reflected by alterations in the structure of the fibroblasts, typified by an increase in cytoplasmic material stainable with neutral red and by an elongation of the mitochondria colorable with janus green This is illustrated in figure 225

*Mast cells* (Germ *masten*, to feed, fatten) are fairly large, plump cells whose cytoplasm is crowded with granules They are not nearly so widely distributed as fibroblasts, but a few can be seen in most spreads of subcutaneous tissue The only cells with which confusion might occur are inwandering eosinophilic leucocytes The cytoplasmic granules of mast cells are generally somewhat smaller and less highly refractile They are soluble in water and usually disappear one-half hour or more after the spreads are made, whereas the eosinophilic granules are very resistant and persist In fixed preparations the mast cell granules are strongly basophilic The nuclei of mast cells are smaller in proportion to the cytoplasm and more spherical than in the eosinophiles—a point that can also be made out by intensive study of fresh spreads, the nuclei being recognized as the part of the cell devoid of granules The mast cells are, as a rule, larger than eosinophilic leuco-



cytes and much less likely to move about between the fibers. Their relation to blood basophiles is uncertain but it is possible that they also produce heparin (p 29) Sylvén (1940) links this idea with the disappearance of mast cells in irradiated tissues

*Macrophages* already have been discussed (p 36) They are known by a host of terms They were referred to by Maximow as 'ameboid wandering cells' and may be regarded as cells of possibly diverse origin which exhibit phagocytic properties The word 'histocyte' should be dropped It is often employed synonymously with fixed macrophages or macrophages which are relatively fixed in position not having enjoyed an opportunity to move about and pick up debris In spreads macrophages differ (1) from the fibroblasts by having smaller nuclei devoid of conspicuous nucleoli which are slightly more refractile because they possess more chromatin and by their more rounded shape (2) from the mast cells and eosinophilic leucocytes by the fact that their cytoplasm is not packed with granules Macrophages are inconspicuous and may be absent in normal tissue They should be studied in subcutaneous tissue from animals which have received several daily injections of trypan blue When this has been done they are highly demarked from the other cells by the fact that they ingest the largest amount of the dye The dye accumulates in the cytoplasm in rounded masses of variable size Fibroblasts take in a little and hold it longer The difference in response to a wide range of dyes is described and beautifully illustrated by Evans and Scott (1932)

The *eosinophiles* are in normal conditions the most constant of wandering blood cells Why they are attracted from the blood stream into the loose connective tissues we do not know They migrate in largest numbers not into the subcutaneous tissue but into the loose connective tissue which underlies epithelia through which there is considerable absorption Thus they line up beneath the epithelial lining of the small intestine and respiratory tract The possibility that they take some part in the detoxification of absorbed substances has been alluded to (p 29) *Lymphocytes* are also of common occurrence They emigrate from the lymphatics as well as the blood vessels both of which are lodged in connective tissue of this type Though lymphocytes vary in size, some of them are the smallest cells encountered in loose connective tissue Enough has been said about them to leave no doubt as to their recognition It is agreed that some of the lymphocytes transform into *plasma cells* which differ from typical small lymphocytes in the possession of rather more cytoplasm This cytoplasm presents the typical lymphocytic basophilia except for a rounded area to one side of the nucleus which remains clear and does not take basic dyes like methylene blue (Figs 62-28) Such cells are frequently encountered in sections of chronically inflamed tissues (cf

#### LEGEND FOR FIG 22

FIG 22a—Structural and functional response of living chicken fibroblasts to various media of different sorts as seen after supravital coloration with janus green and neutral red

13 Plasma growth rate 4.6 14 plasma and embryonic juice growth rate 11.15 plasma growth rate 0.97 16 plasma and embryonic juice growth rate 2.82 17 plasma growth rate 1.2 18 plasma and embryonic juice growth rate 4 19 Tyrode solution growth rate 0.7 20 embryonic juice growth rate 3.28 21 Tyrode solution one stained with neutral red the other with janus green growth rate 1 22 embryonic juice, staining as in 21 growth rate 7.93 Tyrode growth rate 0.01 24 embryonic juice growth rate 2.5 (Carrel and Pridel courtesy of J. Exper. Med.)

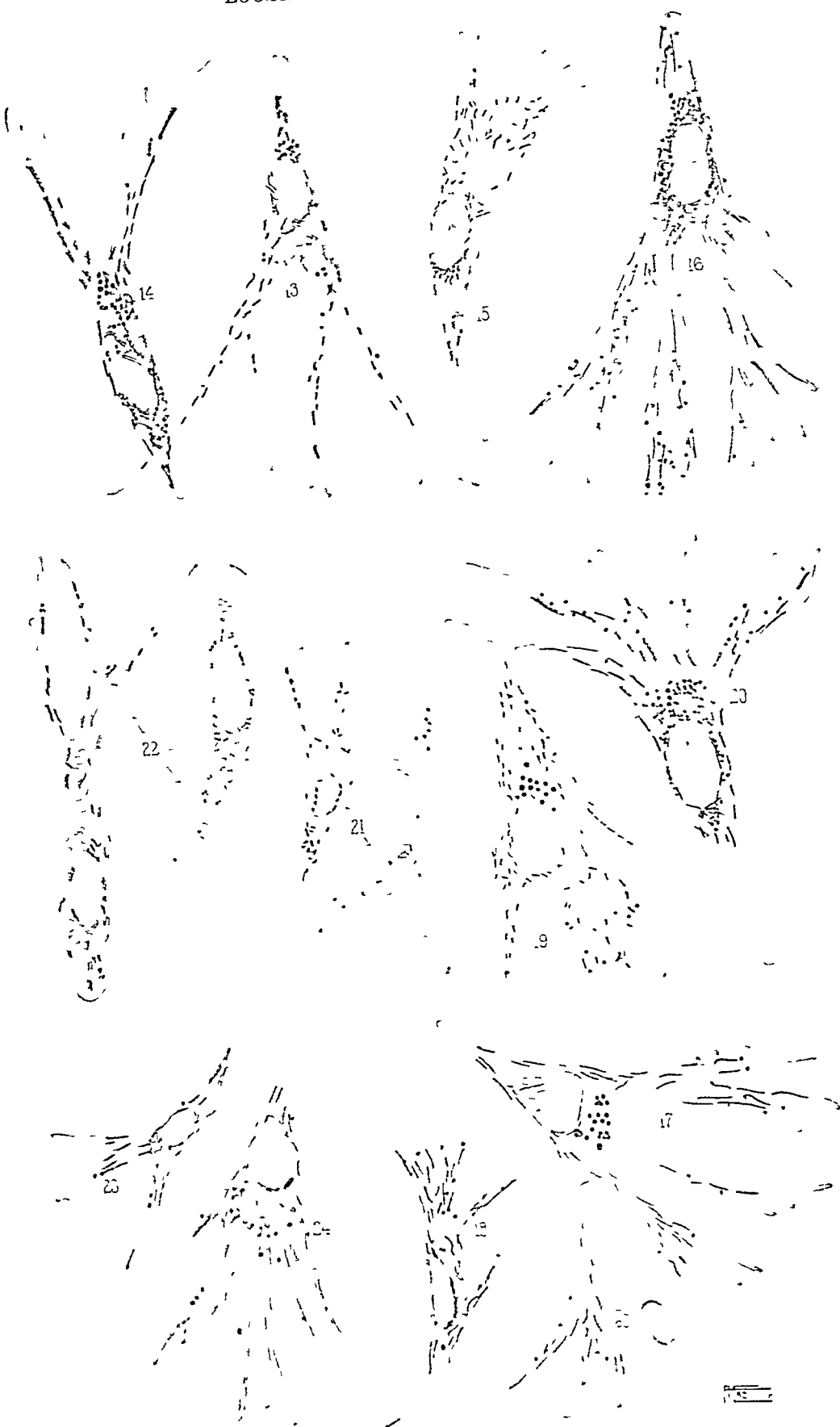


FIG 225

mucosa of nasal sinuses) but it is not to be expected that they will occur with sufficient frequency in spreads of subcutaneous tissue to be easily identified in the fresh state. Sometimes acidophilic *Russell bodies* form in their cytoplasm (Fig. 226). No useful purpose has been ascribed to plasma cells. They are to be looked upon as one of the several end stages in the life cycle of lymphocytes. Other fates of lymphocytes are described on page 31. Polymorphonuclear *leucocytes* only enter the loose connective tissues when they are called for by the penetration of certain bacteria and toxic substances. Red blood cells are found in all spreads of subcutaneous tissue simply for the reason that it is impossible to tear them without rupturing blood vessels. They afford a convenient measure of the

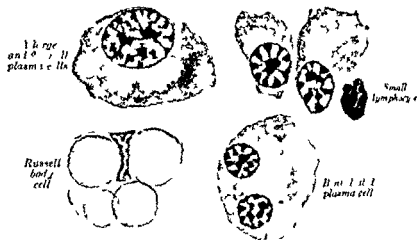


FIG. 226.—Cells from near tonsil. Zenker formol, hematoxylin-eosin stain,  $\times 1000$  (Maximow-Cowdry & Special Cytology, Paul B. Hoeber, Inc.)

Obviously loose connective tissue is very widely distributed. It is spongy but vital because of the contained living cells. If the tissue fluid in it increases because of dilatation of the capillaries and consequent increase in their permeability or because its evacuation is interfered with by insufficient venous or lymphatic drainage, the tissue swells and becomes waterlogged (edematous). The increase in volume may be enormous as in elephantiasis of the legs or scrotum. Water in the loose connective tissue beneath the skin and about the muscles that water is chiefly stored, also glucose and salt. Cannon (1912) speaks of this storage by inundation since there is no chemical change in the material stored. Storage here is a kind of tide that rises and recedes with sufficiency and defec-

In loose connective tissue the ubiquitous *fibroblasts* and the other cell mentioned are relatively fewer per unit volume because of the large amount of tissue fluid and the abundance of fibers than are the cells of epithelial tissue which are closely crowded together. It follows that the metabolic requirements of loose connective tissue are less than those of epithelium. In health a kind of balance is established between the two. But with some injuries the epithelium suffers and the connective tissue whose needs are less is unaffected and may even reverse a phenomenon known as *fibrosis* and manifested by multiplication of fibroblasts and formation of additional fibers. Another explanation is that the connective tissue cells are less highly specialized than epithelial cells and that with generalization in activities the modes of response to injury are reduced in number and ability to multiply in response to it. When specialized cells have to face a

conditions their adjustability is limited in the same manner that an expert may go under when deprived of his occupation and a more versatile man may survive. This argument hinges on the degree of specialization of the fibroblast and upon whether it is actually the fibroblast which multiplies or primitive undifferentiated mesenchymal cells which still lurk in the loose connective tissues and give rise to fibroblasts.

In wound healing loose connective tissue plays a prominent part. Products of cell destruction, which have been unwisely called "necrohormones," stimulate the fibroblasts which multiply, produce collagenic fibers and bind the edges of the wound together. Epithelium, when present, cooperates. The whole problem is well discussed by Arey (1936). Fibroblasts, and the malignant sarcoma cells arising from them, should be demonstrated by presentation of the excellent moving picture films made by Dr. Warren H. Lewis to be rented from the Wistar Institute of Anatomy.

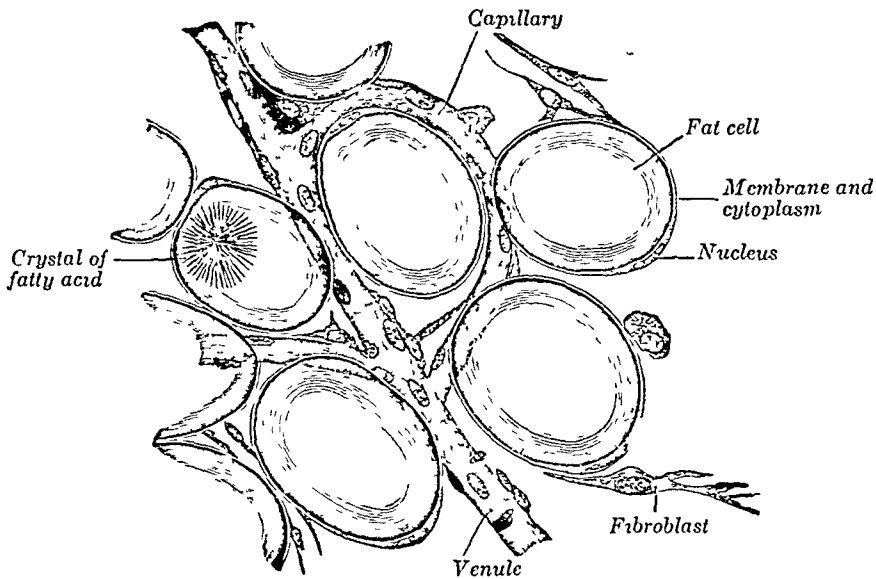


FIG 227 —Cells from the margin of a fat lobule (Sharpey-Schafer, Schafer's Histology, Longmans, Green & Co.)

**Fatty Tissue.**—Fatty tissue differs from loose connective tissue by the fact that the cells have taken in enormous quantities of fat, which stains with Sudan III and blackens with osmic acid. At first sight the cells look like nothing more than droplets of fat. Close examination demonstrates that each droplet is invested by a thin film of cytoplasm which is a little thicker on the side containing the nucleus so that the fat is definitely intracellular (Fig 227).

It is hardly necessary to point out that fatty tissue constitutes an important reserve of energy yielding material, that it provides a convenient packing material of light weight (p. 49), and that subcutaneous fat serves as an insulator against too rapid loss of heat. But investigators have accepted fatty tissues too complacently. Wells (1940) has written a fascinating paper entitled "Adipose Tissue—a neglected subject." That it is a dynamic part of the body influenced by numerous factors concerning which we are densely ignorant is now accepted.

Fat cells are not simply enduring containers with energy-rich material locked up in them at some remote period like the coal strata in a mine. Both microscopic and

chemical evidence show that fat in storage is systematically changed. When cells were held under observation in special chambers inserted in the ears of mice by the Clarks (1940) they were seen to undergo cyclic changes involving accumulation of fat in small droplets, coalescence to form a single large droplet, subsequent decrease in size of this droplet and beginning repetition of the process as illustrated in figure 228. The cycles may be comparatively short in some cases and perhaps longer in others. How often they are repeated by individual cells is unknown, and that Schoenheimer's assumption that fat cells are short lived is unwarranted. Dividing fat cells are scarcely ever seen. The same can be said of dying ones.

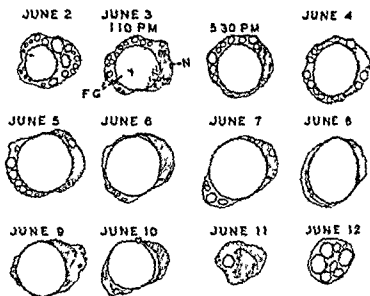


FIG. 228—Camera lucida records of a single new fat cell (Clark and Clark, courtesy of *Am. J. Anat.*)

On the chemical side Schoenheimer (1937) has availed himself of the fact that fatty acids can be marked by inclusion in them of heavy hydrogen (deuterium). In his experiments those in mice held on a carbohydrate diet plus heavy water were replaced in six days by new fatty acids containing heavy hydrogen. This marked a more rapid turn over than that usually taking place, but that it can occur is certain. In another experiment he found that the larger part of dietary fat is oxidized directly but is first deposited in the fatty tissues. From this it seems that nature is a good housewife. She uses up the old food in the refrigerator while storing new food for later service. For further work along this line see Symposium on Intermediate Metabolism of Fats, Biological Symposium Series, C. C. Cattel Press, Lancaster, Pa., 1941.

A perplexing feature of fat cells is absence where not wanted—many in the cutaneous tissue of the abdominal wall, few in that of the scrotum and eyelids, many in cavities of long bones, few within the cranial cavity, etc.

According to one idea fat cells are simply cells originally not characterized by their fatness which have stored fat. Great vagueness is manifest in stating exactly what they were to start with: fibroblasts, reticular cells, or the hang over from mesenchymatous cells which are so convenient to think of and so hard to forget. The presence of much fat in one area and of little or no fat in another could hardly be local differences in the cells in their immediate tissue fluid environments, certainly.

In terms of another view fat cells are a separate type of cell of mesenchymatous lineage characterized by definite properties. But explanation of the occurrence of much and little fat would be in the same terms of local differences in the cells and in their environments

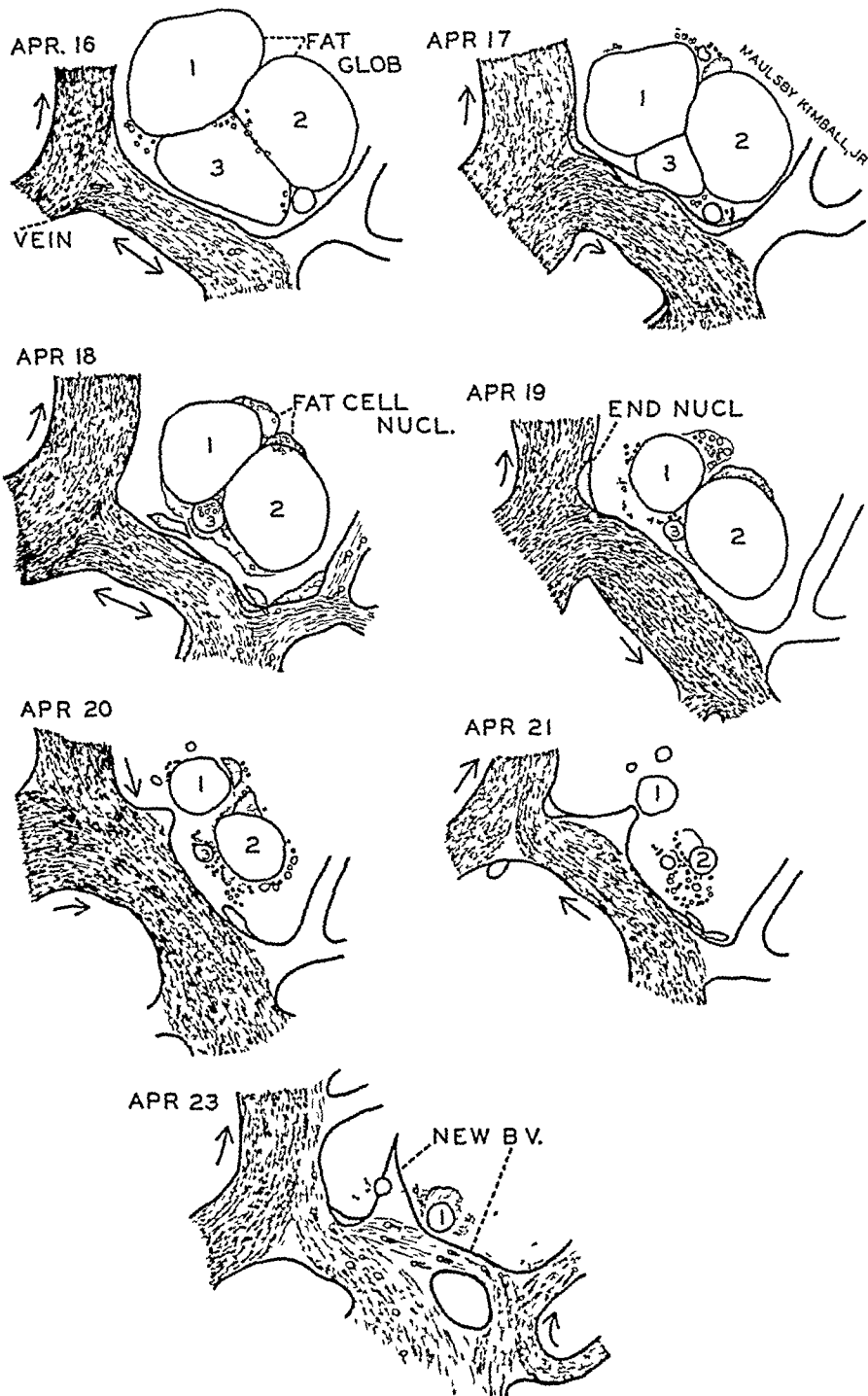


FIG. 229 —Series of records of a group of 3 mature fat cells. (Clark and Clark, courtesy of Am J Anat )

Wells inclines toward the second concept, which is now the most popular, and cites an interesting case. The patient, a girl aged twelve years, lost the skin of the

back of one hand by a severe burn. The surgeon made a graft of skin from the abdominal wall and apparently implanted with the epidermis some subcutaneous fatty tissue. All went well but "as the girl matured and acquired the rotundity of a matron the transplanted skin failed to realize its altered status and laid on the same amount of fat as the original abdominal wall. The result was a grotesque hump-glove effect."

Another example of well established regional difference in behavior of fatty tissues is the acceptance by some of much more fat under the influence of female hormone leading to the development of regional feminine curves. Perhaps these particular fat cells are so turned that they respond to the hormone while others do not or do so to a less extent—a phenomenon sharply demonstrated by epidermal cells (p. 376).

Normally the storage and release of fat is regulated to a nicety in each region, amounts established by custom. If more fat is absorbed than is oxidized it is hoarded up. If oxidation is increased over absorption it is decreased. When the rate of metabolism is geared up by administration of thyroxine stored fat is burned. According to Julian *et al.* (1943) anterior pituitary hormone causes migration of body fat to the liver while lipocortic hormone of the pancreas facilitates movement of fat to body depots. Other hormones may enter the picture as in the special case of activation of the mammary glands after pregnancy. Lipases are involved indeed a host of factors known and undreamed of.

Fatty tissue enjoys a considerable blood supply and the Clarks observed a decrease in the fat content of three cells close to a vessel in which the volume of blood flow increased (Fig. 229). The reverse might perhaps take place if the blood had been charged with fat during absorption from the intestine. It is doubtful whether nerve impulses operate in the storage and discharge of fat except by way of blood vessels.

Heredity is a potent factor in obesity. Wells mentions the occurrence of fatty tumors (lipomas) in all males in two generations. But in such cases the fat is securely locked up in the tumors because the wasting body is unable to withdraw it. Knowledge of this immobilization might go far to explain the mechanisms of normal storage and utilization of fat.

**Cartilage**—In cartilage (I. cartilago, gristle) blood vessels, lymphatics and nerves may be absent. Cartilage is not of fibrous but of hyaline consistency and has a pearly white appearance. In ordinary stained sections the cells seem to be suspended in an optically homogeneous matrix. Only by special techniques can fibers be demonstrated. The ground substance which has been developed by changes in the original tissue fluid between mesenchymal cells is firm but has none of the brittleness of bone. Chemically it consists of chondro-albumoid, chondromucoid and chondroitinsulphuric acid plus the collagen and elastin of the fibers. The affinity of the ground substance for basic dyes is attributed to the acid but Ham (1933) points out that it may be due in part at least to the physical properties of the chondromucoid. Sometimes an acidophilia is noted. This is occasioned by the collagen. How the tissue fluid is altered in this very radical way has never been explained. The true mechanism of almost every process of differentiation eludes us but the formation of cartilage can be watched *in vitro* (Clarks, 1942). After this peculiar matrix has developed the mass of tissue is walled off from the body fluids by a limiting connective tissue membrane, the perichondrium composed of fibrous and collagenic and elastic fibers closely bound together. It is a question whether

some of the fibroblasts of the perichondrium should be dignified by the special term *chondroblasts* to signify their ability to transform into cartilage cells and aid in the formation of new ground substance in regeneration after injury. Certainly the avascularity of cartilage is a factor in the maintenance of its chemical and physical properties. But the cartilage cells are not as isolated as they appear to be. Cytological methods show that they contain typical mitochondria and some glycogen. Fluids bearing oxygen and food supply percolate through the ground substance to them and waste is eliminated by diffusion in the opposite direction. The ageing of cartilage is described by Hass (1943).

Three kinds of cartilage are recognized. *Hyaline cartilage*, as the name implies, is particularly glassy (G *hyalos*, glass) in appearance. It contains fewer fibers than other sorts of cartilage and is, in a sense, more primitive, for in the embryo it formed the basis for the bones of the skeleton (except the membrane ones), which developed from it by calcification. The cartilages of the respiratory tract, which serve primarily to hold the lumen open, belong in this category, also the costal cartilages. The articular cartilages of the joints are also hyaline. In *fibrocartilage* there is a heavy development of collagenic fibers in the ground substance, as in the intervertebral discs and symphysis pubis. *Elastic cartilages* look yellow for the reason that they possess so many yellow elastic fibers. They are more flexible and elastic (epiglottis and certain laryngeal cartilages).

**Bone.**—As a preliminary it is well to note the ways that Nature has solved the problem of mechanical support. Two principal materials have been employed: the carbohydrates, cellulose, in plants, and minerals, especially calcium, in animals. The living cells of plants have not only firm retaining walls of cellulose, for which there is no counterpart in the animal kingdom, but they also contain much fluid. Osmotic forces are harnessed to hold their shape by the operation of an inner plasma membrane comparable to that investing animal cells. Young shoots droop when they die and lose water. The center of a tree trunk may rot and waste away while new cells are formed layer upon layer on the outside. Some cells, however, become dehydrated and the cellulose sets. An old tree holds up its head long after it has died and fluids no longer circulate in its fibrovascular channels.

Both invertebrates and vertebrates have supporting tissues made up of a little protein plus calcium phosphate and carbonate to give firmness, but these constituents are utilized differently. The chitinous investment of arthropods is an exoskeleton that supports and at the same time protects. It is wholly dead, not possessing any living cells or blood supply. The endoskeleton of vertebrates is itself protected from the environment by skin, which is movable on a bed of loose connective tissue supported in some places by fat, so that the force of blows and mechanical injuries is minimized. It is made up of bone on which rigidity is conferred by impregnation with minerals. There is also an organic binding component consisting for the most part of collagen. This has been described in the architecture of teeth. The bones of some vertebrates are hollowed out and filled with air to give lightness. They are, moreover, highly plastic and adaptable to new strains and stresses. In addition to giving support, the bony skeleton serves as a reservoir for that exceedingly important element, calcium. When calcium is needed by the organism it is withdrawn quite rapidly from the bones, and, under certain conditions, is returned to them. Blood formation may and does on occasion occur in many parts of the body, but Nature discovered in the evolution of vertebrates that it was a good plan to seclude it in the most protected parts of the body,



namely within the cavities of the bones. In this way the skeleton developed from the highly talented tissue mesenchyme plays an essential part in integrity by forming blood cells.

Figure 230 illustrates the early development of the leg. First the limb bud is recognizable in 9 mm embryos as a slightly elongated condensation of mesenchyme. This grows until in embryos 11 to 14 mm in length the various segments become distinguishable as masses of cartilage. Between them the mesenchyme undergoes liquifaction and the joints appear in the 20 mm embryo. Then in the 50 mm one the femur is seen to have the cartilage of the outside of the shaft replaced by perichondrial bone (dark stipple).

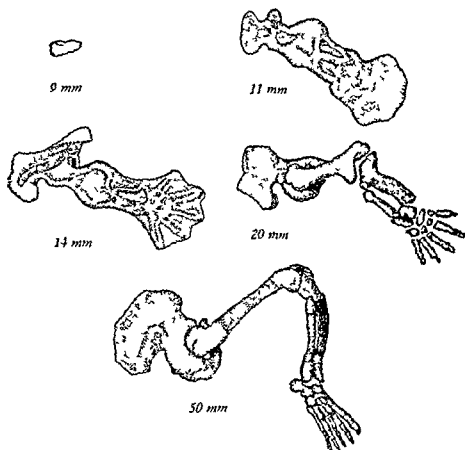


FIG. 230 Stages in embryonic development of the leg. The length of the embryo is given in millimeters. (Redrawn and modified from Bardeen, C. R., courtesy of Am. J. Anat.)

The stages in the development of the femur, beginning in the cartilaginous stage of about a 20 mm embryo are indicated diagrammatically in figure 231. The phases represented are not drawn to scale nor are they accurately dated. Before discussing them it is to be noted that it takes about twenty years to complete the construction of this bone. The increase in length from birth to maturity is about five times and throughout this period the bone must function as a lever—the most powerful one in the body. The task of engineers in producing a lever which does not grow nor adapt itself to changing demands, is childish in comparison.

1. The cartilaginous model of the future bone is illustrated crudely at the extreme left in figure 231. The cartilage is formed from mesenchyme by a process

plication and rounding-up of the mesenchymatous cells, a decrease in blood supply and an increase in intercellular material. This is what is called *interstitial growth*. The vesicular cartilage cells come to be suspended in a firm, slightly elastic, almost hyaline matrix which cuts easily with a knife. The model is surrounded by a con-

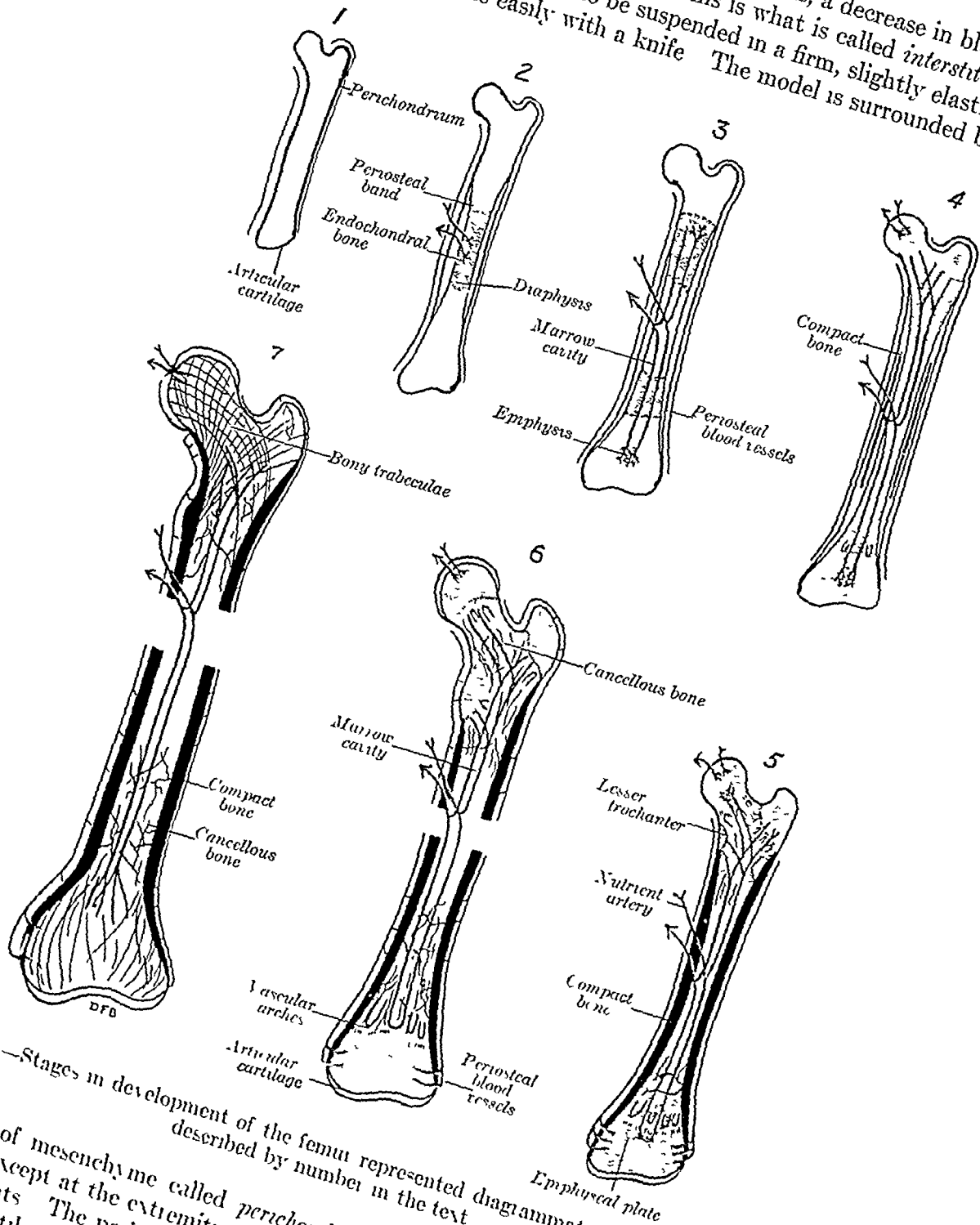


FIG. 231—Stages in development of the femur represented diagrammatically. They are described by number in the text

densation of mesenchyme called *perichondrium* (G *peri*, around, and *chondros*, cartilage) except at the extremities where the cartilage is flattened and lubricated to form joints. The perichondrium is represented by a thick black line and the articular cartilages by thin lines. The vascularization of the perichondrium is

indicated by a red line close to its outer surface but the cartilage within it has no blood vessels at this stage

2 In the perichondrium covering the shaft the closely packed cells are well supplied with materials of many sorts reaching them in the blood stream. They enlarge and begin to deposit intercellular material in thin layers on the surface of the cartilage, and between them which ossifies. They are therefore called *osteoblasts* (*G* *osteon* bone + *blastos* germ) or *osteogenic cells*, because they form bone.

The intercellular material first formed is organic and fibrous. Later on it is impregnated with minerals and ossified. In ordinary preparations the basic organic matrix is masked. More bone is laid down from without some of the osteoblasts become separated from the blood stream, incorporated in the bony matrix and change into bone cells. Osteoblasts are illustrated in figure 230 and bone cells in figure 241.

With this change the perichondrium becomes known as *periosteum* since it invests bone in place of cartilage. The periosteal bone is laid down in a band which encircles the shaft. But the band is very porous so that in many places the vascularized periosteum penetrates in through the bone and comes into intimate contact with the cartilage beneath. Even before the formation of the periosteal bony band the cartilage cells within begin to swell up and the intercellular material to rarefy. Now, blood vessels from the periosteum, accompanied by osteoblasts, actively invade the cartilage in one or more places and they lay down bone in the cartilaginous shaft. This is designated the primary center of endochondral bone formation for it is within cartilage. It is also called the *diaphysis* (*G* *growth* *thru* *h*). The bone thus formed is of spongy consistency since it is deposited by the osteoblasts between and along strands of living cartilage cells. It is indicated by fine stipple in the diagram.

3 At about the time of birth the periosteal band has extended further up and down the shaft. The primary diaphyseal center of endochondral bone formation has likewise extended. At about the middle of the shaft where the large blood vessels enter this bone becomes eroded and resorbed to form the beginning of the marrow cavity. This process of bone destruction is referred to later. At the distal end some blood vessels and osteoblasts from the shaft penetrate deeply into the cartilage. Here a secondary center of endochondral ossification is developed in the same way. It is termed an *epiphysis* because the growth (*physis*) is upon (*epi*) the shaft. The area is represented in fine stipple.

4 Within the periosteal bone of the shaft a layer is differentiated which is known as *compact bone* owing to its hardness. This is indicated by a wide black line. More endochondral bone is formed in the distal epiphysis. In the diaphysis the marrow cavity is extended and endochondral bone encroaches further on the growing cartilages at both ends resulting in an increase in length.

In the proximal end two secondary centers of endochondral ossification appear, the first in the cartilaginous head of the femur during the first year and the second in the cartilaginous great trochanter in the fifth year.

It is important to note that the head with the great trochanter forms one block of cartilage which includes these centers of ossification. Increase in length takes place by a projection upward into this growing cartilage of endochondral bone which replaces the cartilage. An artery, entering with the ligamentum teres, supplies the epiphyseal center of endochondral ossification of the head of the femur and persists in some adults.

5 The endochondral bone spreads to occupy the center of the entire distal end of the femur except for a layer of articular cartilage facing the joint cavity and a layer of cartilage between it and the endochondral bone of the diaphysis. This second layer of cartilage is referred to as an *epiphyseal plate*, or line, of cartilage. It is represented by short, parallel, vertical lines because the cartilage cells in it are arranged in columns (Figs 236-238).

The sides of the distal end are protected by the periosteal bony band. Through this porous band blood vessels enter and these more and more effectively supply the distal epiphysis while those running to it from the diaphysis are reduced. In this periosteal band some compact bone is differentiated.

In the shaft the periosteal band is replaced by compact bone and the marrow cavity is further extended. At the proximal end a third secondary center of endochondral ossification appears for the lesser trochanter. This epiphysis is indicated, like the others, in fine stipple. The endochondral bone spreads upward forming a wedge between the epiphysis of the head and that of the great trochanter, so that the neck of the femur is bony. In the head, all cartilage is replaced by endochondral bone except a layer of articular cartilage and an epiphyseal plate. Endochondral bone in the epiphysis of the great trochanter is spreading.

6 There is great increase in length at the distal extremity by multiplication of cartilage cells and their replacement by bone on the diaphyseal side of the epiphyseal plate. The shaft is wider owing to deposition around it of more compact bone. Some periosteal blood vessels supply blood to the marrow cavity. At the proximal end, the endochondral bone of the epiphyses of the greater and lesser trochanter replaces all the cartilage, except that of the epiphyseal plates, and here also integration by penetrating periosteal blood vessels replaces that from the marrow cavity within. It is at these plates that extension of endochondral bone of the diaphyses takes place. Periosteal bone formation is more active on the proximal (upper) side of the neck than on the lower side.

It has been repeatedly demonstrated, when metal pegs are inserted a measured distance apart in the shaft and after growth in length has taken place, that the distance between them remains the same. When, however, one peg is placed in the shaft and the other in an epiphysis, they separate as the bone increases in length. This proves that it is in the epiphyseal plates of cartilage, which intervene between the secondary endochondral ossification centers (epiphyses) and the primary center (diaphysis), that growth takes place. In these plates of young animals the cartilage cells can be seen in the process of multiplication. The growth is said to be appositional because the cartilage cells are changed into bone and are added to the bone at the ends of the shaft. This is to be contrasted with the interstitial growth, or internal growth, by which expansion of the original cartilage model is brought about.

When a metal band is fitted closely about the shaft of a young bone, after the bone as it grows increases in girth, the band is discovered free in the marrow cavity. This proves that, through the activity of the periosteum, more and more compact bone is deposited on the outer surface while, with increase in size of the marrow cavity it was removed from the inside.

An excellent way to identify newly formed bone is to feed a young animal madder. This dye is deposited in the intercellular material of ossifying bone as long as it is being offered to it in the blood stream. When the bones of small animals are cleared it is seen that the bone laid down during this period is colored bright

crimson (see Macklin 1917a). The U.S.P. unit of vitamin D potency is calculated from the colored line (Lane test) of matrix produced under influence of vitamin alizarin sulfonate in animals whose diet is supplemented with measured amounts of the vitamin as compared with controls (see Martin 1940). Interesting experiments are being made with radio-strontium which accumulates in the epiphyseal plates and in bone tumors where it can be demonstrated by radioautography (Trendelenburg *et al.* 1942). This may be a step on the high road toward treatment.



FIG. 232.—Lines of arrested growth in the distal end of the tibia. A. Roentgenogram of right ankle joint of a girl aged four years and ten months. Analysis of clinical history showed that the lines corresponded with severe illnesses. B. Three years and five months later. The original lines appear to have moved up the tibia by the deposition of new bone at the extremity. This deposition also is marked by definitely spaced lines of arrested bone related in time to other illnesses. (Harris, Brit. J. Radiol.)

Lines of arrested growth which are more compact than usual can be recognized in roentgen ray photographs (Harris 1933). Two of these are presented in Figure 232. The first (A) was taken of the lower end of the tibia and fibula of a child at the age of four years and ten months. The lines of arrested growth appear dark and their formation is correlated with a perfectly definite series of severe illnesses when growth had to be sacrificed in the struggle for survival. The second (B) shows the same bones at eight years and three months. The lines previously recognized are apparent situated farther away from the end of the bone for new bone with other lines of arrested growth has been added. These synchronized with attacks of

bronchopneumonia and bronchiolectasis. Such experiences are indelibly written in the growing bony skeleton.

Examination of the diagram, figure 231, especially of stages 5 and 6, shows that the endochondral bone added to the diaphysis at the distal epiphyseal plate is wider than the diameter of the bone proximally. This means that the bone once formed is reshaped by a reduction in girth. At the proximal end there is more extensive remodelling. Figure 233 indicates the shape that the head of the femur would assume in growth by extension from five to fifteen years of age if remodelling did not take place.

7 With final cessation of growth in length at the epiphyseal plates the endochondral bone of the epiphyses joins that of the diaphysis. This union of epiphyses takes place in definite order, those first to appear join first, the last of them about the twentieth year. The actual observed time of union of various epiphyses is made by Todd (1933) the basis of an interesting and valuable determination of physiological age. Retardation of growth as well as precocious growth can be accurately measured in this way.

When union has been accomplished the bone is solid from the proximal to the distal articular cartilage. How these vestiges of the original cartilage model are regularly, and exactly to the same degree, exempted from ossification is a mystery. The costal cartilages attached to the ribs are similarly immune until late in life when calcification in them is patchy.

The structure of bone is very well adapted to its use. Bone may be likened to reinforced concrete insofar that the organic fibrous material of the matrix gives reinforcement and the calcification cohesive strength. But it is dynamic and highly adaptable not static. Changes in functional demand are followed by definite alterations in internal structure and external configuration in accordance with mathematical principles. Koch (1917) has made a detailed mathematical analysis of the femur. Figure 234 indicates how closely the trabeculae of cancellous bone and the thickenings of compact bone correspond to lines of mechanical strain. Ample factors of safety, providing ordinarily unnecessary strength, are supplied in order that it will not fail in emergencies.

Now for the details. First the *epiphyseal plates*. Figure 235 shows a section of the distal end of the femur and the location of figures, 236, 237 and 238. It is essential to remember

(1) That the cartilage of the plate intervenes between the endochondral bone of the epiphysis

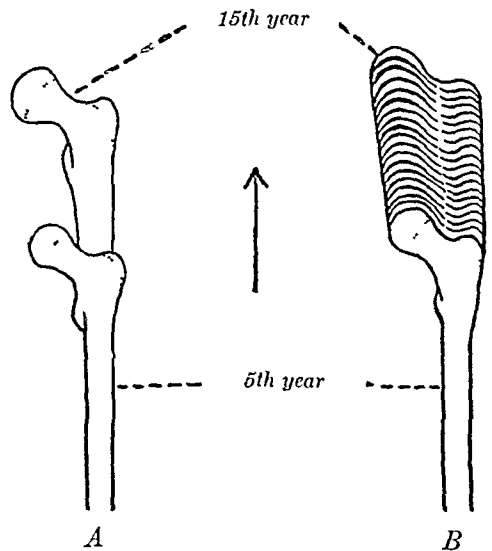


FIG 233 —A, upper end of femur of five-year child, above which is indicated the position which the head and the neck of the bone will occupy at fifteen years. B, the form which it would have assumed had there been no remodelling. The arrow indicates the direction of appositional growth. (Keith, Sir Arthur, *The Engines of the Human Body*, courtesy of Williams and Norgate, London.)

crimson (see Macklin 1917a). The I S P unit of vitamin D potency is calculated from the colored line (Line test) of matrix produced under influence of vitamin D alizarin sulfonate in animals whose diet is supplemented with measured amount of the vitamin as compared with controls (see Martin 1940). Interesting experiments are being made with radiostrontium which accumulates in the epiphyseal plates and in bone tumors where it can be demonstrated by radioactive light (Ireadwell, *et al* 1942). This may be a step on the high road toward treatment.



FIG. 232—Lines of arrested growth in the distal end of the tibia. *A* Radiograph of right ankle joint of a girl aged four years and ten months. Analysis of clinical history showed that the lines corresponded with severe illnesses. *B* Three years and five months later. The original lines appear to have moved up the tibia by the deposition of new bone at the extremity. This deposition also is marked by definitely spaced lines of arrested growth related in time to other illnesses. (Harris Brit J Radiol)

Lines of arrested growth which are more compact than usual can be recognized in roentgen ray photographs (Harris 1933). Two of these are presented in figure 232. The first (*A*) was taken of the lower end of the tibia and fibula of a child of the age of four years and ten months. The lines of arrested growth appear dark and their formation is correlated with a perfectly definite series of severe illnesses when growth had to be sacrificed in the struggle for survival. The second (*B*) shows the same bones at eight years and three months. The lines previously recognized are now apparent situated farther away from the end of the bone for new bone with other lines of arrested growth has been added. These synchronized with attacks of

bronchopneumonia and bronchiolectasis. Such experiences are indelibly written in the growing bony skeleton.

Examination of the diagram, figure 231, especially of stages 5 and 6, shows that the endochondral bone added to the diaphysis at the distal epiphyseal plate is wider than the diameter of the bone proximally. This means that the bone once formed is reshaped by a reduction in girth. At the proximal end there is more extensive remodelling. Figure 233 indicates the shape that the head of the femur would assume in growth by extension from five to fifteen years of age if remodelling did not take place.

7. With final cessation of growth in length at the epiphyseal plates the endochondral bone of the epiphyses joins that of the diaphysis. This union of epiphyses takes place in definite order, those first to appear join first, the last of them about the twentieth year. The actual observed time of union of various epiphyses is made by Todd (1933) the basis of an interesting and valuable determination of physiological age. Retardation of growth as well as precocious growth can be accurately measured in this way.

When union has been accomplished the bone is solid from the proximal to the distal articular cartilage. How these vestiges of the original cartilage model are regularly, and exactly to the same degree, exempted from ossification is a mystery. The costal cartilages attached to the ribs are similarly immune until late in life when calcification in them is patchy.

The structure of bone is very well adapted to its use. Bone may be likened to reinforced concrete insofar that the organic fibrous material of the matrix gives reinforcement and the calcification cohesive strength. But it is dynamic and highly adaptable not static. Changes in functional demand are followed by definite alterations in internal structure and external configuration in accordance with mathematical principles. Koch (1917) has made a detailed mathematical analysis of the femur. Figure 234 indicates how closely the trabeculae of cancellous bone and the thickenings of compact bone correspond to lines of mechanical strain. Ample factors of safety, providing ordinarily unnecessary strength, are supplied in order that it will not fail in emergencies.

Now for the details. First the *epiphyseal plates*. Figure 235 shows a section of the distal end of the femur and the location of figures, 236, 237 and 238. It is essential to remember

(1) That the cartilage of the plate intervenes between the endochondral bone of the epiphysis.

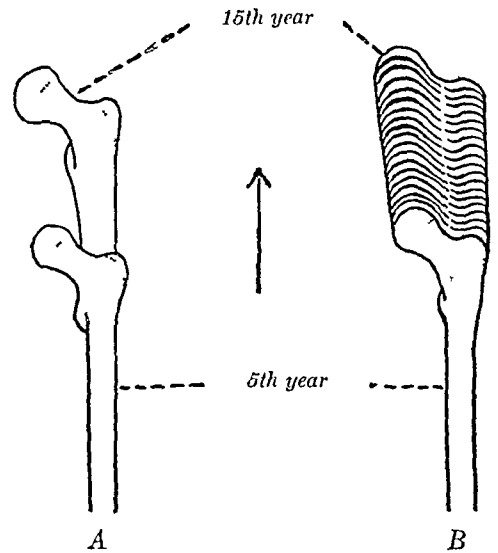


FIG 233 —A, upper end of femur of five-year child, above which is indicated the position which the head and the neck of the bone will occupy at fifteen years. B, the form which it would have assumed had there been no remodelling. The arrow indicates the direction of appositional growth (Keith, Sir Arthur, *The Engines of the Human Body*, courtesy of Williams and Norgate, London.)



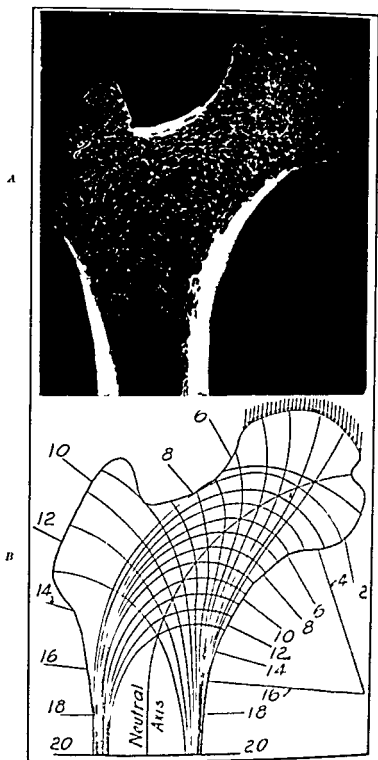


FIG. 34. Demonstration of relation of bony architecture to lines of stress in the femur. *A* The actual structure. Note the heavy compact bone of the sides of the shaft and arrangement of bony trabeculae in the cancellous part. *B* Mathematical analysis of lines of stress resulting from load on head of femur indicated by arrows. (Koch: *Am. J. Anat.*)

(2) That the cartilage cells multiply and become arranged in columns disposed roughly parallel to the direction of growth (consult Dodds, 1930 and Ham, 1931).

(3) That the growth pressure leads to a flattening of the cells

(4) That for some reason, not understood, the cartilage cells next the epiphyseal bone remain dormant and applied more or less immovably against it (Fig 237), whereas those on the other side, near the diaphysis, become hypertrophied and the intercellular substance between them less dense

(5) That osteoblasts, accompanied by blood vessels, bore into this degenerating cartilage from the diaphysis

(6) That these osteoblasts excavate tunnels in it and, following their usual custom, lay down bony matrix on the cartilaginous sides of the tunnels (Fig. 236).

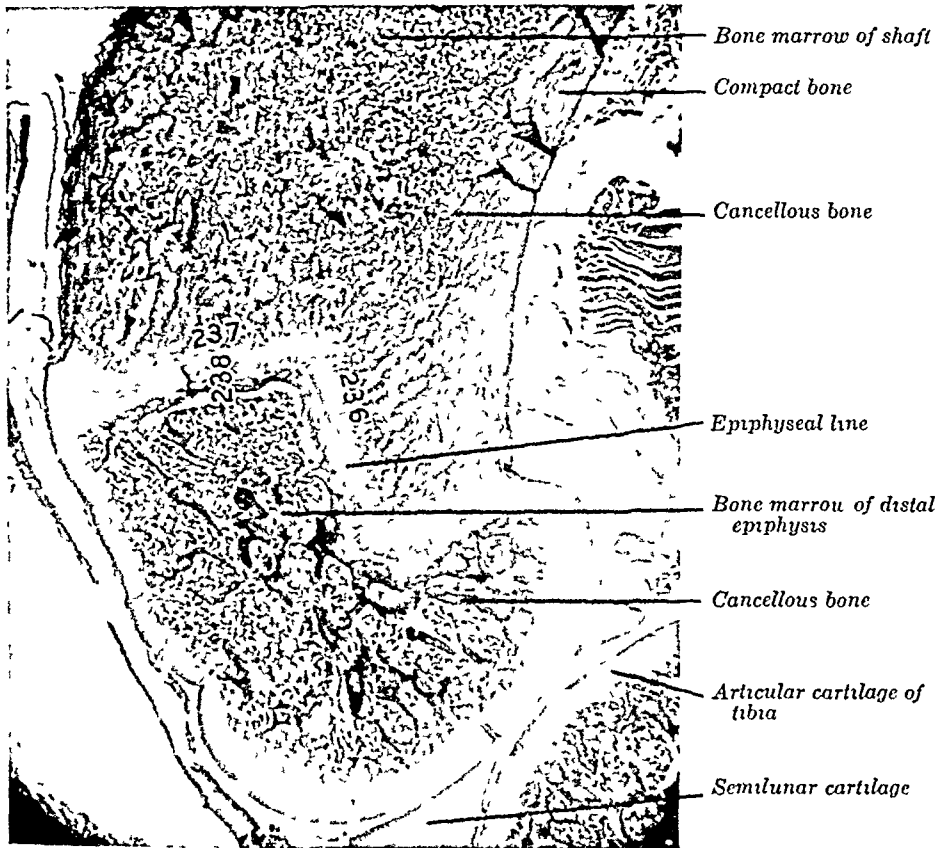


FIG 235 —Longitudinal section of lower end of femur of a guinea-pig The location of figures 236–238 is indicated (Photomicrograph by Dr Arthur Ham)

(7) That by this means more and more cartilage, as it is formed, is replaced by spongy, cancellous bone.

(8) That this cancellous bone, being added by appositional growth to that already present, causes increase in length of the central part of the bony shaft while the bony epiphysis, which backs up the expanding epiphyseal plate, moves farther and farther away.

(9) That this extension continues until the cartilage cells stop multiplying, all undergo degeneration and the cancellous bone, which replaces them, unites with the bone of the epiphyses Where necessary, for mechanical reasons, layer upon layer

of bone is laid down within the tunnels forming Haversian systems and leads to the development of compact bone as described later (p. 202).

How bone is actually constructed is not well known. The hypertrophied cartilage cells and the osteoblasts may elaborate an enzyme phosphatase (see Huxley, 1932), which acts on the combined phosphate (hexosephosphate) in the matrix of the cell, freeing the phosphate ions so that they are added to those already present in the tissue fluid. They are able by mass action to cause the precipitation of calcium phos-

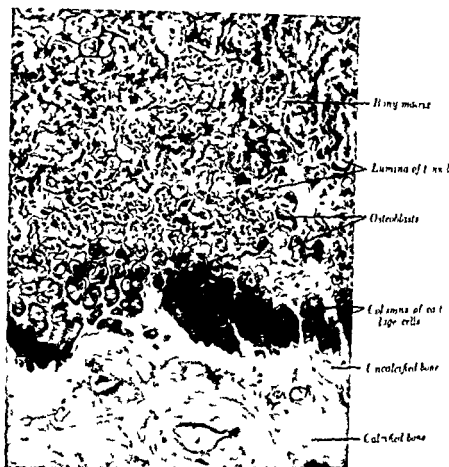


FIG. 236.—Section of area marked in Figure 235. Epiphysis below and diaphysis above. Note passing upward (1) calcified bone of epiphysis (2) uncalcified bone of epiphysis (3) cartilaginous tunnels forming by the disintegration on the distal side of the plate of cartilage cells stacked one upon another in columns like a series of coins (4) darkly stained osteoblasts which line the inner walls of the tunnels and lay down bony matrix which many of them have already laid down in the interiors of the tunnels. (Photomicrograph by Dr. Arthur Ham.)

phate which precipitate because of the physico-chemical attraction of bone and cartilage matrix settles into the organic intercellular substance. This mechanism provides a means whereby a precipitate will form in the region of bone and not in the blood stream or in other normally uncalcified tissues. The precipitate is not pure calcium phosphate. Carbonate ions are also present at the site of the precipitation hence a complex carbonate and phosphate of calcium forms. This is called carbonato-apatite.

The orderliness of normal bone development is impressive. Each kind of bone grows according to its own schedule which involves a delicately regulated balance between bone formation and bone resorption, between simultaneous deposition of calcium in some parts and removal of calcium from others. Bone possesses an inherent capacity for growth and differentiation that is not dependent upon the particular tissue environment in which it normally develops. Willis (1936) trans-

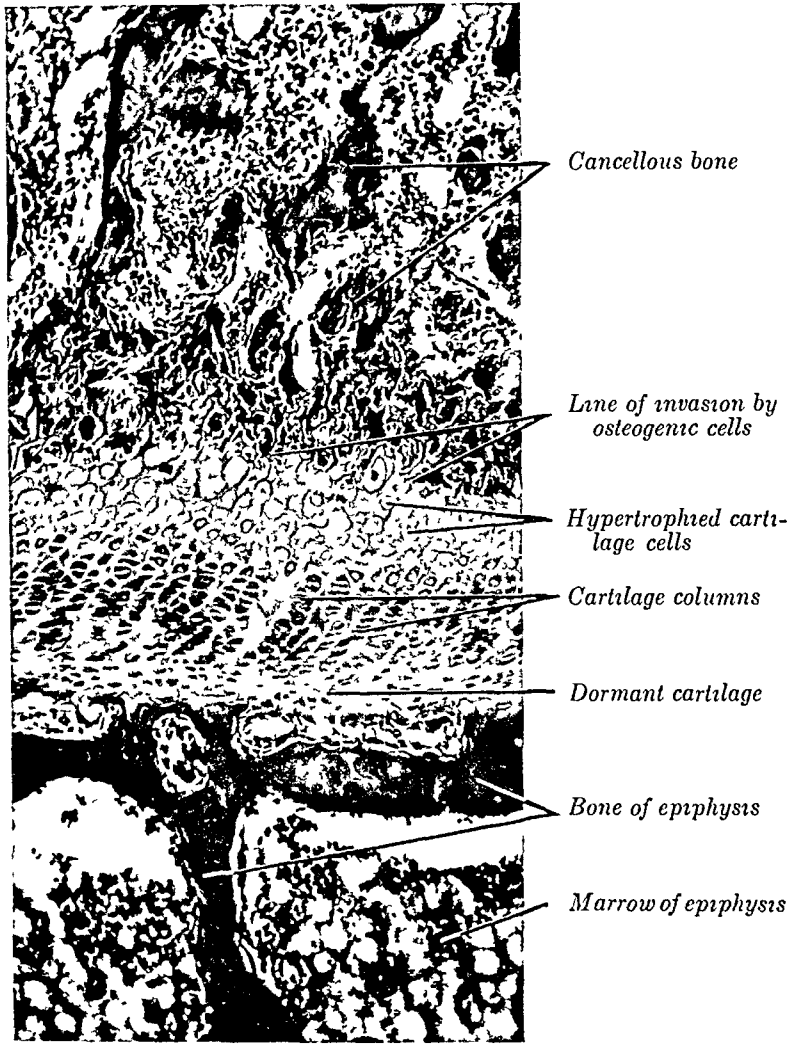


FIG 237 —Longitudinal section of area marked in Figure 235 in a normal rat on the left. Epiphysis below and diaphysis above. Note passing upward (1) marrow of epiphysis, (2) bone of epiphysis, (3) narrow layer of dormant flattened cartilage cells lightly stained, (4) deeply staining columns of cartilage cells, (5) band of hypertrophied cartilage cells, (6) invasion of osteogenic cells and capillaries, (7) formation of cancellous bone. (Photomicrograph by Dr. Arthur Ham.)

planted cartilaginous limb buds into the brains of young rats. These developed femurs with articular surfaces, epiphyseal plates and bone marrow. The remarkable bilateral symmetry in ossification, as Pryor (1936) points out, is evidence of equal supply of the required hormones, vitamins and other substances *via* the blood stream.

Deviations from normal growth are worthy of close attention. Use leads to strengthening and disuse to atrophy of bone. Achondroplastic dwarfs, as the name

suggests suffer from lack of moulding of bone in cartilage, the cartilage cells in the epiphyseal plates of their long bones go on strike but periosteal bone continues to be laid down on the shaft. Consequently the bones of arms and legs become thin and thick. These unfortunates were the court jesters in ancient times. Even more tragic is excessive activity of the cartilage cells of the plates and invading osteoblasts usually about puberty causing increase in length (and growth) of bones far beyond normal. Such individuals are called pituitary giants because at least one of the factors is pituitary hypersecretion.

Three dietary disturbances of the epiphyseal plates are represented in figure 238. When children receive insufficient calcium and vitamin D, they become "rickety" and are said to suffer from rickets. The cartilage cells of the plates continue to multiply but replacement by extension of endochondral bone from the diaphysis is inhibited. The width of the plates is increased and on the sides of the bone they may bulge slightly so that they can be felt as rings between the shafts and ends of the bones.

Vitamin C deficiency tends to inhibit the osteoblasts and prevent the formation of bone matrix. Heavy dosage with parathormone withholds the necessary calcium so that connective tissue is formed in place of endochondral bone. In the clinical condition of hyperparathyroidism, the bony changes are similar but more chronic in nature (osteitis fibrosa). The bones are calcium reservoirs. When more than the usual amount of calcium is needed, as in pregnancy, the bones lose calcium become painful and osteomalacia results (*G. osteon, bone + malacia softness*). In a rapidly growing animal the cartilage cells of the epiphyseal plates are multiplying actively. For this reason (and perhaps as a consequence of comparative avascularity) they are particularly sensitive to roentgen ray. Strong radiation will kill them and bring about premature union of epiphyseal and diaphyseal bone causing arrest of growth (Regen and Wilkins 1936). Bony changes from youth to old age are beautifully illustrated by Amprino and Bairati (1935).

Compact bone can be examined unstained in pieces made thin by grinding (Fig. 239) or in sections but after decalcification and stained in the usual way (Fig. 240). As already mentioned compact bone is formed in the periosteal band. The osteoblasts on the surface of the band lay down *circumferential lamella* of bone. Some periosteal vessels run almost parallel with the length of the bone in grooves.

#### LEGEND FOR FIG. 238

FIG. 238 — Longitudinal sections of area marked in figure 235 but from experimental rats. Epiphysis on the left and diaphysis on the right. Above: From rat rendered rickety by low P, high Ca and vitamin D deficient diet. This inhibits normal calcification of cartilage of epiphyseal plate; the cartilage cells live long, tongues extending into diaphysis. Some cartilage, however, is replaced by uncalcified bone which is feebly stained as seen in lower right hand corner.

Middle: Similar area from guinea pig held for two months on diet containing only a one-half normal vitamin C requirement. Since this vitamin is necessary for excellent activity of osteoblasts (and odontoblasts of teeth) these cells are greatly reduced on the diaphyseal side of the epiphyseal plate and the formation of bone matrix has almost ceased.

Below: Similar area from young guinea pig injected forty-eight hours previously with a large dose of parathormone. The bony trabeculae normally located on diaphyseal side of epiphyseal plate are absent; their place being taken by young connective tissue and a mass of large, darkly stained osteoclasts. (Photomicrographs by Dr. Arthur Ham.)

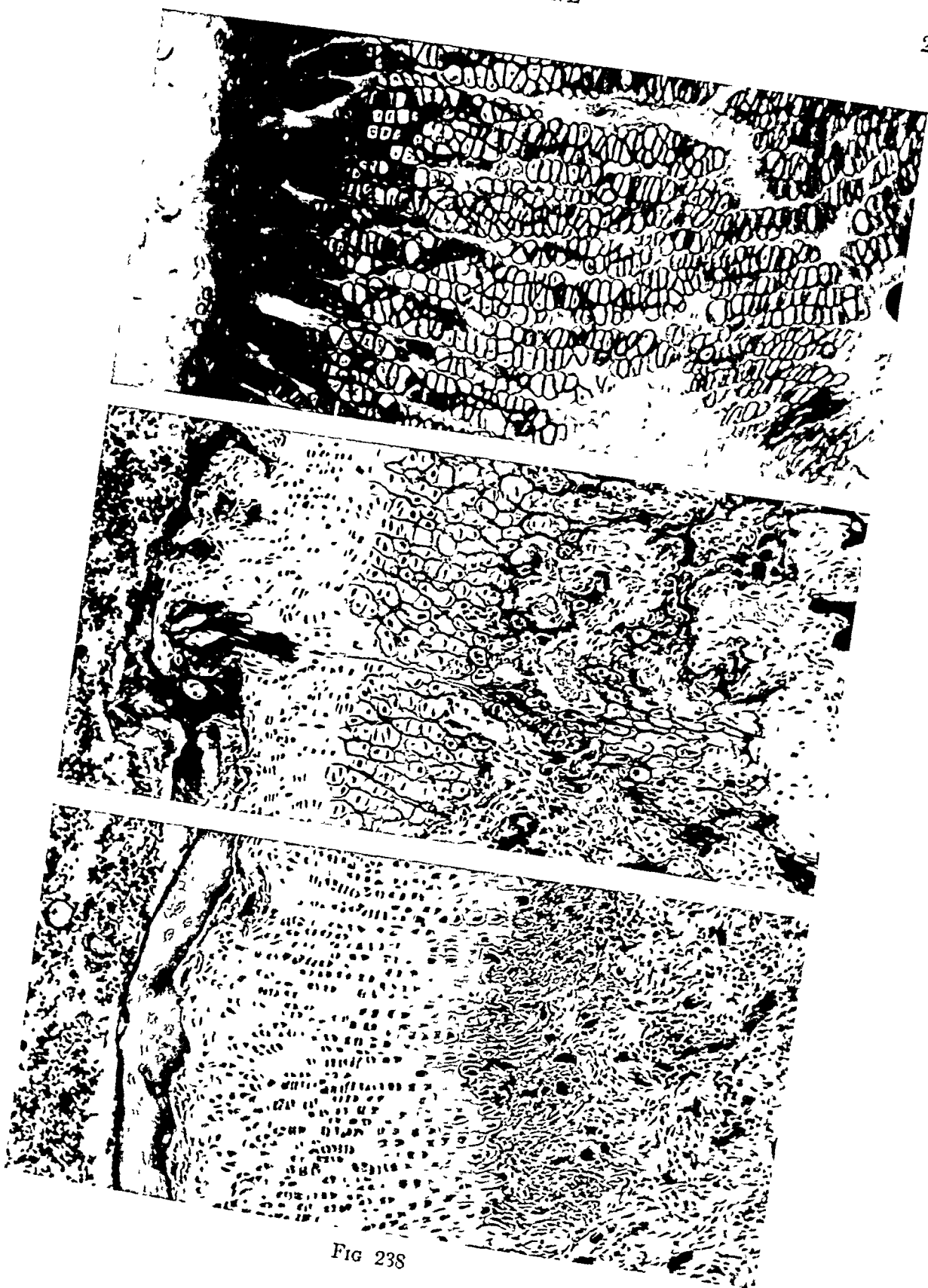


FIG 238

These are covered by the circumferential lamellæ and constitute little finger-like projections of the osteoblastic sheet. The vitality and the integration with the rest of the body of each projection is maintained by an artery, a nerve, a return vein and a draining lymphatic. These projections, with the bone which they form

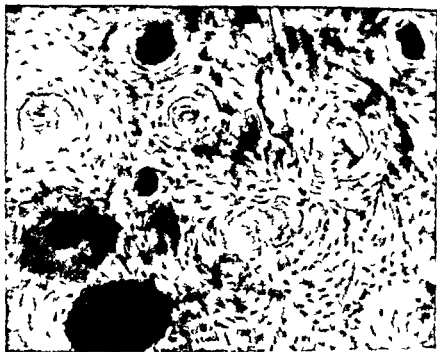


FIG. 239 — Cross section of ground compact bone of dog demonstrating Haversian systems with surrounding lamellæ.  $\times 150$  (Ham Cowdry's Special Cytology, Paul B. Hoeber, Inc.)



FIG. 240 — Cross-section of radius of dog, decalcified specimen. Haversian systems may be seen, the former showing an osteoblastic cell lining and the latter (Ham Cowdry's Special Cytology, Paul B. Hoeber, Inc.)

are designated *Haversian systems*. They are easily recognized in decalcified bone (Fig. 240) by the centrally placed artery and the *concentric lamellæ* laid down one after another by layer upon layer of osteoblasts developing intercellular material and changing into bone cells. The Haversian systems ordinarily run more or less parallel with the long axis of the bone so that, in cross-sections of bone, they are cut transversely. As the lumina of the Haversian systems are narrowed by the formation of concentric lamellæ, the bone becomes compact. At the same time the circumferential lamellæ between the Haversian systems become displaced, broken up and irregular and are then known as *ground lamellæ*.

*Volkmann's canals* are generally of larger lumina, carry vessels destined for the Haversian systems, are seldom surrounded by lamellæ, enter the bone almost perpendicular to the surface and are therefore seldom cut transversely in cross-sections.

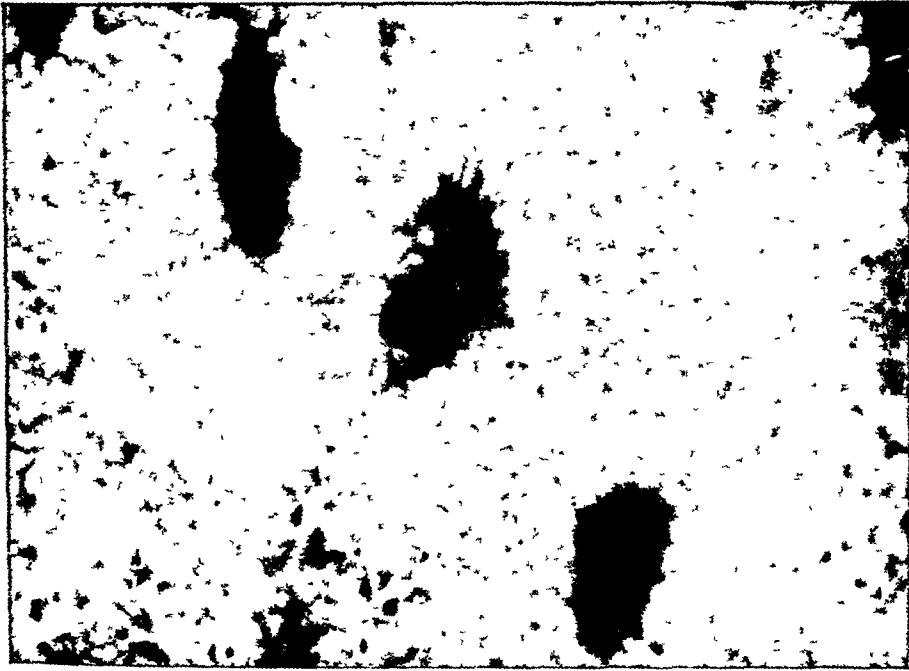


FIG 241.—Lacunæ and canaliculi of bone of dog. Decalcified section. Giemsa stain  
X 1500 (Ham, Cowdry's Special Cytology, Paul B. Hoeber, Inc.)

The fact that the lumina of these canals are not narrowed, in the same fashion as those of the Haversian canals by deposition of bone, is worthy of emphasis. The delicate tubes by which blood vessels pass through the epiphyseal plates to supply the developing endochondral bone of the epiphysis are held open until another supply is established from the investing periosteal bone. After this some may still remain. Obviously bone is normally formed only where it is needed, not where its presence would curtail the blood supply.

The *osteocytes* or bone cells, into which the osteoblasts transform when they surround themselves with a calcified fibrous matrix, continue to live. They are lodged in tiny bony cavities called *lacunæ* (L. dim. of *lacus* a hollow) and they extend out delicate protoplasmic processes in bony canaliculi. These cells are seen as black masses rather evenly spaced in concentric lamellæ in figure 239. They are represented at high magnification in figure 241.

Bone is developed and maintained by wonderfully regulated and localized processes of formation and resorption. As we have seen,



(1) That endochondral bone is developed during growth on the diaphyseal sides of the epiphyseal plates

(2) That approaching the diaphysis this bone becomes excavated and cancellous the trabeculae being oriented along lines of stress (Fig. 234)

(3) That in the center of the diaphysis the same bone is completely resorbed to make way for the marrow cavity

(4) That periosteal bone is developed on the surface and within embedded Haversian systems

(5) That compact bone of periosteal origin is at the same time removed by resorption in the diaphysis particularly at about its center

In other words the organic framework, calcium and other minerals are being deposited in some places synchronous with their active removal from others. Bone resorption is as interesting as bone development. If we could fully understand one the other would soon be made clear. Examination of trabeculae of cancellous bone being reabsorbed to make way for the marrow cavity, and the inner surface of the wall of the bony shaft of a long bone reveals an incomplete single layer of cells called the *endosteum* in contrast to the *periosteum* without. Some may be osteoblasts capable of forming bone. Others are large cells which pass under the heads of osteoclasts (*G* = osteon, bone + *klastos*, *klaos* = I break) on the theory that they function in the removal of bone. Support is lent to this idea by the fact that each osteoclast occupies a little hollowed out part of bony wall (a Howship's lacuna). An osteoclast is called a "giant cell" on account of its large size and a polynuclear because it contains many nuclei. The nuclei are spherical or oval in shape and colored quite intensely with hematoxylin. The cytoplasm is acidophilic, whereas that of neighboring osteoblasts is basophilic. Ham (1932) remarks that the osteoclasts look as if they had contracted in the course of fixation for they are seldom in actual contact with the bone or neighboring cells. Instead they are surrounded by a vacant area across which a few strands of their cytoplasm are extended. The osteoclasts do not take up tripan blue (Shipley and Macklin 1916; Cappel 1930) but phagocytose bone cells (Arey 1917) and other materials (Jordan 1920-21). Haggqvist cites experiments and reasons for thinking that the osteoclasts are to be regarded as foreign body giant cells developed in the vicinity of bone (foreign body) by fusion of osteogenic cells. The latter are relatively undifferentiated and present in the vicinity in large numbers. A novel idea has been advanced by Haggqvist (1938) who suggests in a splendidly illustrated paper that the osteoclasts result from the confluence of bone cells freed by the absorption of bone around them. Howell (1890-91) and Arey are among the few who do not ascribe absorption of bone to the osteoclasts. The work of Hofmeister (1910) indicates that any metabolically active cell in contact with bone will liberate  $\text{CO}_2$  and cause the calcium salts to go into solution. It is very likely therefore that while the osteoclasts are not specifically concerned in bone resorption they do, with the other cells that may be present aid in the process. The mechanism of removal of the organic matrix has been insufficiently studied. Macrophages may help to do so.

Moving away from the surface into the substance of the bone matrix various stages in the development of blood cells are encountered which have been mentioned (p. 200). In addition there are *megakaryocytes* which are giant cells with large nuclei and therefore easily recognized.

In the healing of fractures there is first a hemorrhage from the torn blood vessels into the fracture area. Some of the tissues including the bone adjacent to the lesion

of fracture, die. The dead tissue and extravasated blood incite an inflammatory reaction which soon passes into the phase of repair. Forty-eight hours after the injury, mitotic figures are evident in the inner layer of the periosteum near the break. This layer of cells steadily proliferates for days and even weeks and so lifts the outer, fibrous layer of the periosteum away from the shaft (Fig. 242, *F*). This results in the formation of fusiform enlargements about each fragment near the line of fracture, which swell, flow over the break, meet and fuse. Continuity between the periosteum of two fragments is established.

While the majority of the cells of the inner periosteum are multiplying some differentiate into bone (*N B.*) and others into cartilage (*C*). The cells of the endosteum, within, also proliferate and form cartilage and bone. After the two fragments have been thus united a process of remodelling is instituted. This is concerned with the replacement of the cartilage by bone in a manner identical with that observed in the development and growth of bone, and with a gradual resorption of the trabeculae more distantly located from the shaft and a reinforcement of those situated close to the shaft. Eventually all the dead bone at the site of fracture is resorbed and the original line of the shaft is almost perfectly restored by new bone.

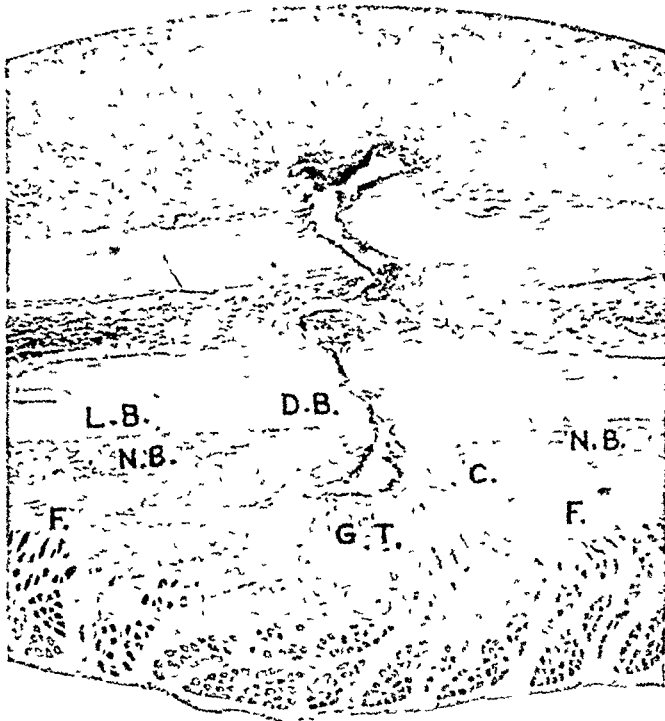


FIG 242 —Healing fracture of rabbit's rib, five days' duration. *L B.* living bone, *D B.* dead bone, *N B.* new bone, *C.* cartilage, *G T.* granulation tissue, and *F* fibrous layer of periosteum (Photomicrograph by Dr Arthur Ham)

Occasionally fractures do not heal properly and the fragments become joined together by nothing but fibrous connective tissue. After certain measures taken to produce bony union have failed, and in certain other cases where a considerable amount of regeneration is desired, *transplants* or *grafts* of living bone are sometimes employed. What happens in these transplants and whether they can be considered as true grafts is controversial. But when pieces of bone are sawn out of a shaft and then replaced in the same location which they formerly occupied and are examined at intervals the following changes are observed:

1 A gradually developing degeneration and necrosis of many of the adult bone cells of the transplant

2 The osteoblasts of the periosteum and endosteum of the transplant continue to live, and proliferate and form new bone

3 Those of the host also proliferate much as they do in repair of a fracture and give rise to a great deal of new bone which unites either with the new bone formed from the transplant or with the original bone of which it was composed whether it is dead or living

Thus at least a portion of a transplant inserted under favorable conditions into the tissues of the same animal or person from which it was obtained may serve as a graft. Even if the transplant were dead and unable to act in this way it would still be useful as a bridge between the two pieces of bone and as a stimulus to osteogenesis. But calcium phosphate and carbonate do not of themselves stimulate osteogenesis when implanted in a bone defect (Key 1934)

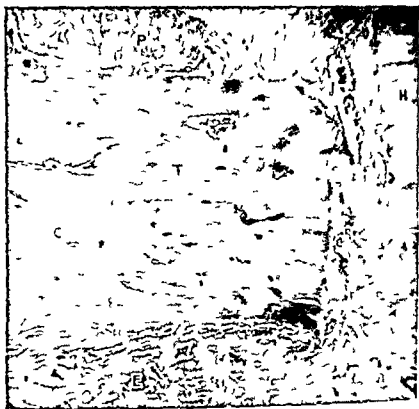


FIG 243 Transplant of cortical bone complete with periosteum and endosteum which has been in place in defect in dog's radius for two weeks. T transplant H bone of host P periosteal new bone E endosteal new bone (Photomicrograph by Dr Arthur Ham)

Figure 243 is a photograph of a simple transplant which was taken from the radius of a dog and immediately put back in place after making sure that it was completely detached from all tissue connection and blood supply. It was in place two weeks before it was recovered and sectioned. It is apparent that the osteoblasts of the graft have grown and formed a knob of tissue (I) and that the graft is firmly united to a great deal of new bone which formed in the narrow cavity

from the endosteum of the host and possibly from the endosteum of the graft. Much of the bone of the transplant remains alive, other parts of it are dead and the dead parts are being dissolved away and replaced (on the right) by new bone.

We have dealt chiefly with long bones preformed in cartilage because they are more easily described. Irregularly shaped bones, like the scapula and the vertebræ, are essentially similar. The bones of the vault of the skull are, on the contrary, called *membrane bones* because ossification takes place in a membrane, or sheet of mesenchyme, and not by invasion and replacement of cartilage by osteoblasts. Membrane bone formation is illustrated in figure 244. As we have emphasized, a fibrous organic matrix is elaborated which becomes stiffened by mineralization.

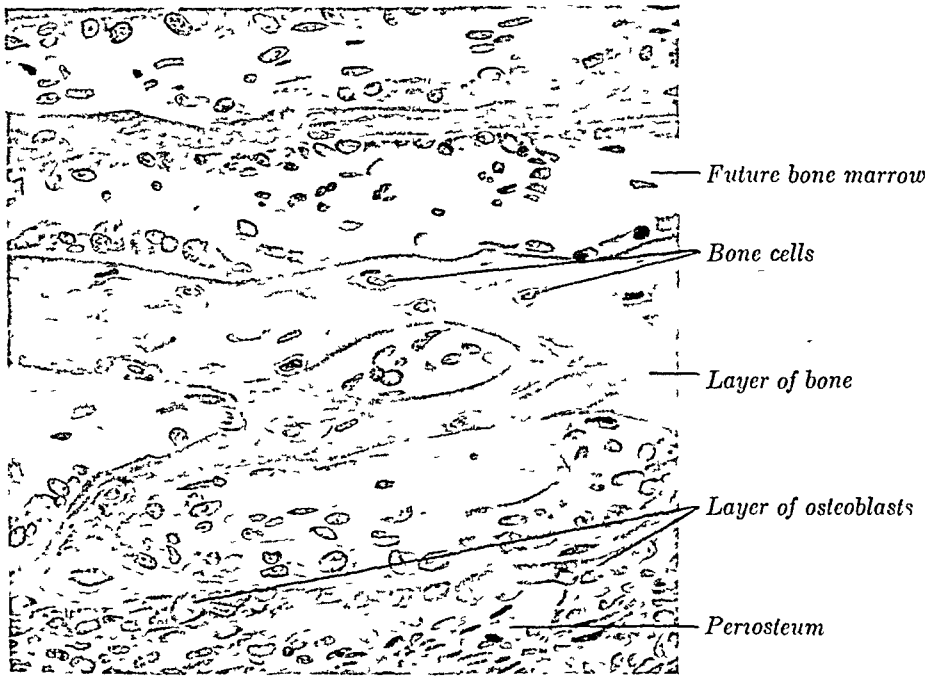


FIG 244 —Membrane bone formation Skull of opossum.

Mesenchyme cells, fibroblasts of the periosteum and osteoblasts (or osteogenic cells), wherever they may be situated, are of the same lineage and not sharply defined cell types. They are capable of many changes including transformation into cartilage cells and osteocytes. Wherever primitive mesenchyme persists (and it is difficult or impossible to clearly identify as such), or fibroblasts occur, bone can form if conditions are favorable. For example, bone, even equipped with bone marrow, has been observed to develop in the walls of the aorta and in many other places. The phenomenon is designated *metaplastic ossification* (G. *metaplasia*, transformation), for the tissues undergo a bony transformation in locations where bone is not needed and where they ordinarily do not do so.

Finally, it is interesting to contrast the hard tissues of the body:

**Articulations.**—Joints are divided into two great groups: *synarthroses* (G. *syn*, together + *arthrosis*, articulation) in which the bones are united by ligaments, and *diarthroses* in which they are separated by fluid. The parieto-occipital suture is an example of the first and the shoulder joint one of the second. Further classification of varieties is the task of the gross anatomists. We are here primarily concerned with the histological details of a diarthrodial joint.

## COMPARISON OF ENAMEL, DENTIN AND COMPACT BONE

	Enamel	Dentin	Bone
Consistency	Hardest	Intermediate	Least hard
Inorganic component	98 to 99 per cent	About 72 per cent	60 to 70 per cent
Organic component	1 to 2 per cent keratin	28 per cent. Collagen yields gelatin on boiling	30 to 40 per cent. Collagen with traces osseonuclein as osseocalciumoid yield gelatin on boiling
Origin	Ectodermal germ-blasts	Mesenchymal odontoblasts	Mesenchymal osteoblasts
Cells	Absent	Absent only Tomes fibers	Present osteocytes
Organization	Rods	Tubules	Lamellae
Blood vessels	Absent	Absent	Present
Nerves	Absent	Present	Present
Lymphatics	Absent	Absent	Present in Haversian canals
Regeneration	Nil	Only as secondary dentin in pulp cavity	In healing of fractures
Fate of dead	Functionally mechanically	Still serves in devitalized teeth	Removed or treated as a foreign useless body

The opposed surfaces of the two bones are capped with hyaline articular cartilage which when lubricated with synovial fluid permits of movement with very little friction. Smoothness is combined with a certain yielding elasticity which is advantageous. Near the surface the cells are somewhat flattened and spread out in layers but as the subchondral bone is approached they become arranged in columns. Between them the glassy looking but fibrous intercellular material gives great resilience. The fibers are continuous at the margin with the collagenic fibers of the capsule. No other material in the body is more subject to jolts and blows. It is of necessity avascular.

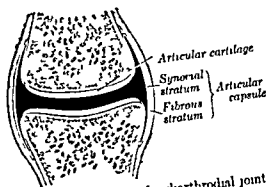


FIG. 245.—Diagram of a diarthrodial joint (Gray's Anatomy)

A segment of pliable rubber hose equipped with well-developed retaining fibers (collagenic) to prevent undue stretching. The inner surface of this cylindrical investment is further supported in some places by ligaments. It may be perforated into the joint cavity in the form of rills or exaginated between nerves, tendons or muscles and bone as bursa which are fluid-containing sacs that ex-

## ARTICULATIONS

the strain. The inner surface of the capsule and of the villi and bursæ is limited by what is termed a *synovial membrane* (G *syn*, together + L *ovum*, egg), the properties of which are best described by Sigurdson (1930) and Key (1932). The membrane is areolar, fibrous or adipose in nature, depending upon local require-



A Normal



B Slight inflammation



C More inflammation

FIG 246 —Shows rearrangement of mesenchymatous cells of synovial membrane in slight inflammation. A, Normal. Cells scattered somewhat flattened parallel to surface and exhibiting Golgi network irregularly placed. B, Slightly inflamed. Cells increased in number. Those next the surface are arranged in an epithelium-like layer with Golgi networks restricted to the poles of the cells next the surface. C, More inflamed. Surface is uneven, some cells have entered the lumen and others are binucleated. (Redrawn from King, courtesy of Jour Path and Bact.)

ments, and is not bordered internally by a special layer of mesothelium as in the serous cavities. Instead, the surface facing the fluid within the joint is merely constituted of connective tissue fibers and cells (fibroblasts) firmly bound together and supplied with blood vessels, lymphatics and nerves (Gardner, 1942). Interesting changes occur in inflammation (Fig 246). Developmentally, the joint cavity

is a space in the primitive mesenchyme that has formed between bones where freedom of movement has by millions of years of evolution become hereditarily established. It is walled in at the sides by a condensation of mesenchyme.

*Synovial fluid* is a thin glairy liquid like the white of an egg. It is almost acellular. Coggeshall, Warren and Bauer (1940) only found an average of 63 cells per cmm in aspirated synovial fluids from 29 normal human knee joints. Of these 1 per cent were listed by them as mononuclear phagocytes, 21.6 per cent lymphocytes, 6.5 per cent polymorphonuclear leucocytes, 4.3 per cent synovial cells and 2.2 per cent unidentified cells. Key (1925) noted that after death the fluid is quickly invaded by leucocytes so that the total count increases in six hours to over 500 per cmm. Resorption of small molecules is chiefly by blood directly at all but lymphatics.

Any concept of the structure of diarthrodial joints based upon the hurried examination of a few sections is bound to be inadequate. Students therefore are advised carefully to examine the excellent series of plates provided in Bennett, Wayne and Bauer's (1942) monograph on 'Changes in the Knee-joint at Various Ages'. In this gross and microscopic findings are correlated. No better illustrations can be given of the structural adjustments to long continued but interrupted mechanical stresses and strains. Wonderful use is made by Nature of non-living materials. In mice at any rate there is evidence of differences in hereditary endowment (Silberberg's 1941).

### SUMMARY

The system of connective tissues of mesenchymatous origin presents more local specializations than any other in the body. This is probably because it contains three different components—tissue fluid, fibers and cells. The first is relatively large in volume and, not being directly swept onward by the blood, has a chance to develop peculiar modifications conditioned by local environments. To handle on a large scale the functions served requires long and careful thought and, since outlook is shaped by experience, no two people will emphasize exactly the same attributes. But some functions leap to the eye, as for example, integration by the mechanical association of parts and, conversely, the separation and segregation of activities by the construction of capsules and membranes so that the environment for each may be undisturbed and adapted to its needs. Mechanical devices providing for rigidity, tensile strength and elasticity are constructed largely of mammoth material which is fairly durable. Freedom of movement is provided by the formation of smooth surfaces and special lubricants. Loose connective tissue, at hand almost everywhere, plays an important part in defense of the body against infection in wound healing and in storage of water, salt and glucose. Potential energy is stored in adipose tissue. Calcium with phosphorus and other minerals in exactly the right proportions accumulate in bones which partly relinquish them in old age when there is a remarkable and massive shift of minerals to the walls of blood vessels, often making the vessels easily visible on x-ray examination. Because the system contains so many non-living components, metabolic requirements are less than those of more cellular tissues. Among these components collagenic (+ reticuli) and elastic fibers are of great importance. Their quality, formation and replacement are as yet little known. Connective tissue cells inhabit the fluid environment of great diversity and adjust themselves without apparent difficulty, but the mitotics among them may become malignant and throw off community control.

## CHAPTER XVII

### MUSCULAR SYSTEM

MUSCULAR movement, the most evident sign of life, has received close attention. Over 2000 years ago Aristotle described the process of flexion and its rôle in locomotion in terms that are acceptable today. But why the shortening of certain tissues, easily seen and felt beneath the skin, came to be called *muscular* contraction is not clear. Derivation of the term from *musculus*, Latin diminutive of the word, *mus*, a mouse, is considered probable by Fulton (1926). To liken the rippling muscles, even of an athlete, to movements of little subcutaneous mice must, however, have required a lively imagination.

A discussion of skeletal muscle naturally follows an account of bones and joints. We have met with muscles that are unconsciously regulated in several parts of the body and the movements of cilia, of individual cells and even of materials within cells, have been briefly described. Motility is not only a fundamental attribute of all living things, but also, in the physical world, motion of some sort is universal. In this chapter the four types of muscle are described together, for much is to be gained by contrasting their distinctive form and function.

To examine muscle in section is not sufficient. The popular techniques of vital and supravital staining are not of much help. The constituent cells are tightly bound together and difficult to separate for study in physiological saline solution at high magnification. It is necessary to resort to the almost forgotten art of maceration by which the connections between the muscle cells are dissolved. For this purpose place small pieces of the wall of the uterus (or stomach), a limb muscle and heart muscle in 10 per cent hydrochloric acid in physiological saline for one to two days. Pour off the solution, wash gently in water and try to separate the muscle cells.

**Smooth Muscle.**—In some respects this muscle is the most primitive. It is called non-striated, plain or involuntary, because it does not have the cross-striations found in skeletal muscles which are under voluntary control.

The structural units of smooth muscle are long, spindle-shaped cells each of which possesses a single rather pointed nucleus at about its middle where its girth is greatest. The cytoplasm, in fixed and stained preparations, shows a faint longitudinal striation attributable to intracellular fibrils—the myofibrils.

Pull is not exerted at the fine ends of the cells, but at the sides, which are closely bound together by reticular fibers so that muscular strands and sheets are formed made up of many cells. These are not inserted by tendons into bones, but are built into the walls of blood vessels and hollow viscera. The circular and longitudinal layers, in which they are commonly arranged, are apt to have a spiral tilt.

Smooth muscle is generally innervated by both sympathetic and parasympathetic fibers. The nerve endings are simpler and less complicated than those in skeletal muscle. Vasoconstriction and vasodilation already have been described (p. 64). All smooth muscles hold a certain degree of contraction, called *tonus*, for a long time and in an untiring way. Movements are involuntary, often rhythmic. Contraction bands are areas of shortening, which extend somewhat irregularly



across muscles made up of many fibers and which stain more strongly than the uncontracted parts. As seen in the inner circular and outer longitudinal muscle of the colon they are illustrated in figure 134 (p. 173). The nuclei also appear to be more deeply colored in these bands. In the walls of small arteries the nuclei are often folded and twisted in consequence of contraction of the investing cytoplasm. Details are described and well illustrated by Roskin (1936). Blood supply is meager compared with that of skeletal muscle.

## COMPARISON OF TYPES OF MUSCLE

	<i>Smooth</i>	<i>Skeletal</i>	<i>Cardiac</i>	<i>Involuntary</i>
Distribution	Digestive respiratory circulatory and urogenital systems spleen large lymphatics, corium endocardium etc.	Attached to skeleton	Myocardium	Myocardium and endocardium
Shape	Long tapering cells rarely branching at ends bound together by adhesion of lateral surfaces	Same as smooth	Short branching with blunt ill-defined extremities arranged end to end to form a practically continuous network	Same as cardiac but usually a bit more robust
Size	Length 15-500 $\mu$ diameter 2-20 $\mu$	Length 1000-20000 $\mu$ diameter 10-150 $\mu$	Accurate measurements not feasible	Accurate measurements not feasible
Cell membrane	No definite sarcolemma	Forms sarcolemma	Sarcolemma less marked than in striated	Sarcolemma slightly developed
Intercalated discs	Absent	Absent	Present	Present
Nuclei	Single occupying middle of cell	Multiple usually peripheral	Generally 1 or 2 situated in middle of cell seldom peripheral	2-3 or more in center of cell
Myofibrils	Non-striated almost fill the cell	Striated almost fill the cell	Striated almost fill the cell	Striated confined to peripheral parts of cytoplasm
Sarcoplasm (non fibrillar)	Small amount	Small amount	Larger amount	Larger amount
Blood supply	Poor	Rich	Twice that of skeletal	Rich arteries and to be territorial
Control	Chiefly involuntary but in rare cases may be at will	Voluntary	Involuntary	Involuntary

Because smooth muscle cannot conveniently be obtained in large quantities for chemical analysis and is not so temptingly arranged for physiological study as

## SKELETAL MUSCLE

investigation has been rather neglected in favor of skeletal muscle. An important property is the ease with which it undergoes hypertrophy. The extent of the change in individual cells during pregnancy is indicated in figure 247. It has been proved that, in the uterine hypertrophy of castrated animals caused by estrogen injections, there is a great increase in number as well as in size of the fibers (Barks and Overholser, 1938). Cellular multiplication is by mitotic division and can be produced by mechanical distension of the uterus as well as by hormones (Allen, Hisaw and Gardner, 1939).

The Macklins (1942) refer to the hypertrophy of smooth muscle in the walls of bronchioles, which produces such distressing symptoms in asthmatics owing to constriction of the lumina. Since these symptoms do not tend to become less severe in old age, we would not expect the hypertrophy to be any less in older than in younger persons.

But more serious is the disastrous hypertrophy of smooth muscle in the arteriolar walls of hypertensives. Here the change appears to be due to direct action on the cells of a pressor substance producing vasospasm (Keyser and Keeton, 1941) and the evidence of permanency is more convincing.

Much may be gained by a detailed comparison of the factors operating in these three situations. Many questions leap to mind. What, for instance, are the chemical and structural differences between smooth muscles in which the hypertrophy is transitory and permanent? Is cell division involved in the bronchiolar and arteriolar hypertrophies? What are the significant differences between these hypertrophies and the localized ones in smooth muscle tumors? Is there any way of making the permanently hypertrophied muscles show in the old age the atrophy so characteristic, as we shall see, of striated muscle?

Haggqvist (1931) has reported that he could find no observations in the literature on age changes in smooth muscle and that his own studies were negative. Todd concluded in 1939 (see Todd, 1942) that "it is certain that smooth muscle preserves its youthful character even in advanced age." But there is some reason to think that smooth muscle "does fail with age, losing the fine edge of its tonus as the years roll by" and that this is partly secondary to autonomic nerve decline (Macklins, 1942).

**Skeletal Muscle.**—"Skeletal" is a more distinctive term than "striated" because cardiac muscle is also striated. It also places these muscles as the movers of the skeleton. But this is not their sole function for some are inserted in the skin while others serve as voluntary sphincters. The designation "voluntary muscle" is also satisfactory. As insisted by Schiefferdecker (1927) muscles of this sort exhibit a measure of individuality in structural pattern. He has compared 116 of them. We

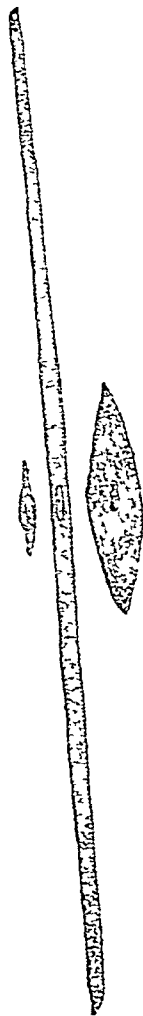


FIG 247—Difference in size of smooth fibers from origin, pregnant and puerperal uteri (Redrawn from Stander, 1936, after Stieve, J W Williams, Obstetrics, D Appleton Century Co, 1936)

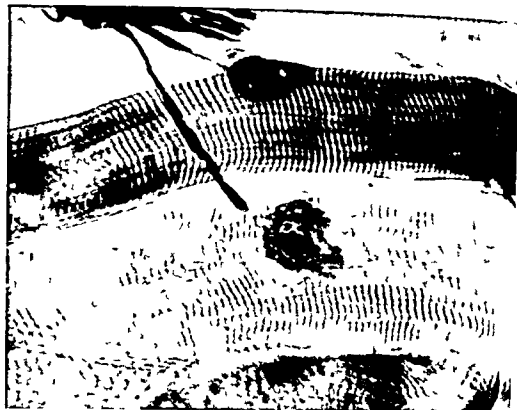


FIG. 248. *Above* Motor end plates prepared by gall chloride technique in a rat intercostal muscle of rat.  $\times 750$ . The upper horizontal fiber is relaxed; dark area possesses a retracted motor end plate. While the lower one is less intensely stimulated; a more expanded motor end plate.

*Below* Similar preparations made after stimulation for one minute with CO<sub>2</sub>.  $\times 750$ . The motor end plates are expanded in both dark and light muscle fibers as compared with those shown above. (From Carey, courtesy of Am. J. Path.)

recall the individuality among arteries not only in structure but in responses to unfavorable conditions

The structural unit is the fiber, really a large multinucleated cell of a length measurable in cms and of girth grading almost up to naked eye visibility (say  $100\ \mu$ ) These fibers are the largest cells in the body and they have a very tough cell membrane, known as the sarcolemma When they are torn this membrane may stand out apart from the cell contents as a homogeneous looking, elastic sheet of appreciable thickness Evidently the plasma membrane, that limits the cytoplasm of all cells, is here greatly reinforced The sarcolemma is not, like the neurilemma, a membrane produced by the flattening of special cells applied to the surface of the fiber Skeletal muscle fibers are bound together by connective tissue to form primary, secondary and even tertiary bundles

The most outstanding features of skeletal muscle, easily seen in stained sections, are the peripherally placed nuclei and the cross-striations The former are illustrated in both longitudinal and transverse sections of tongue muscles in figure 111 (p 151) The latter are well shown in figure 248



FIG 249 —Photomicrograph of incinerated longitudinal section of rectus abdominis muscle of cat Cross-striations are marked by large mineral residue of Q discs which appears white when viewed in the dark field  $\times 3150$  (Photomicrograph by Gordon H Scott)

The dark bands are stained more darkly in most preparations than the light ones They are doubly refractile, birefringent or anisotropic (*G anisos* unequal + *tropos* a turning), for when viewed in polarized light (with crossed nicols), they turn the rays of light and shine brightly white, whereas the light bands, being isotropic, remain invisible Both bands are to be regarded as segmental differentiations, or discs, in myofibrils that are closely packed side by side in the fiber in such a way that the dark bands and light bands are abreast of those in neighboring fibers Certain transverse lines can also be made out, which, like the discs, have been called many names so that the terminology is confusing and complicated Suffice it to

say that the dark bands masquerade as  $I$  and  $Q$  discs and the light ones as  $I$  and  $J$  discs

Studies on the muscle protein myosin have been summarized by Partridge (1942). In the dark bands needle-like molecules of this material are believed to be oriented parallel to the length of the fiber and to condition their birefringence while in the light bands they are disposed at random. That the mineral content of the two bands is different can be seen by micromineralization (Scott 1932) and by use of a special electron microscope (Scott and Packer, 1939). Employment of secondary magnification up to 23 000 is described by Richards *et al* (1942).

The alternating dark and light bands are so conspicuous that it is not surprising that many attempts have been made to explain their genesis. Carey (1940b) is of the opinion that the cross-striations are to be considered as hydraulic pressure waves radiated from the motor nerve ending and from liquid molecules or crystals which have been aligned by the tension or stretching of differential growth. This theory is difficult to prove particularly when one tries to understand operation of the hypothetical pressure waves in striated muscle tumors as productive of striations about central foci like those illustrated by Iwing (1940). That the cross-striations once formed can change has been discovered by Carey and associates (1942a) in his study of the influence of increase in temperature on their number and pattern in single living nerve fibers.

The highly viscid consistency of muscle substance was first demonstrated by Kite (1913) in his pioneer microdissections of living muscle cells and has since been confirmed by de Renvy and Hogue (1934).

When the fibers are macerated in dilute acid and teased apart the delicate individual myofibrils which are cross-striated can be distinguished. That these exist *in vivo* can be inferred from Hogue's (1937) observation of distinct myofibrils with cross-striations in bundles and singly in living contracting cardiac muscle cells which are thinner and more easily examined at high magnification than the similarly striated skeletal muscle fibers.

Actual stages in the development of cross-striations have been observed in living tadpoles by Speidel (1938) whose illustrations should be examined. It is remarkable at what speed the nuclei divide, cross-striations appear and the nuclei take up a peripheral position. But in cardiac muscle cells (Goss 1940) and presumably in skeletal ones myofibrils and cross-striations are not developed until a few hours after contractile activity is well established. Goss has expressed the opinion that these cytological differentiations are less associated with contraction than with a harnessing of contractile force in order to exert mechanical pull on the cell.

Not all of the substance of skeletal muscle fibers is made up of closely packed myofibrils and nuclei. There is also present some less differentiated cytoplasm at the poles of the nuclei and to a less extent between the myofibrils. The ubiquitous mitochondria are always to be found in it with variable amounts of glycogen, lipid pigment, muscle hemoglobin and other substances. In fact skeletal muscle is sometimes divided into red and white varieties as in the dark and light meat of chickens. The former are made up of fibers of smaller girth, contain more nuclei, granular cytoplasm and muscle hemoglobin and are less distinctly striated and less susceptible to fatigue. One would suspect that the red muscles would have a larger blood supply but they are reported (Stoel 1924) as having less.

man, striated muscle is usually regarded as a mixture of both types, the soleus being more red than the gastrocnemius (see Fulton's 1926 discussion).

The phenomena of muscular contraction are properly left to the physiologists. It is sufficient to remember that there is a reversal of striation. The dark band that stains deeply in the relaxed state takes the stain less intensely and *vice versa*. Influence of tetanizing on the bands has been studied by Meneely (1939) and is illustrated in figure 250. Speidel's (1939) fine description of microscopic changes in contracting single fibers provides many details.

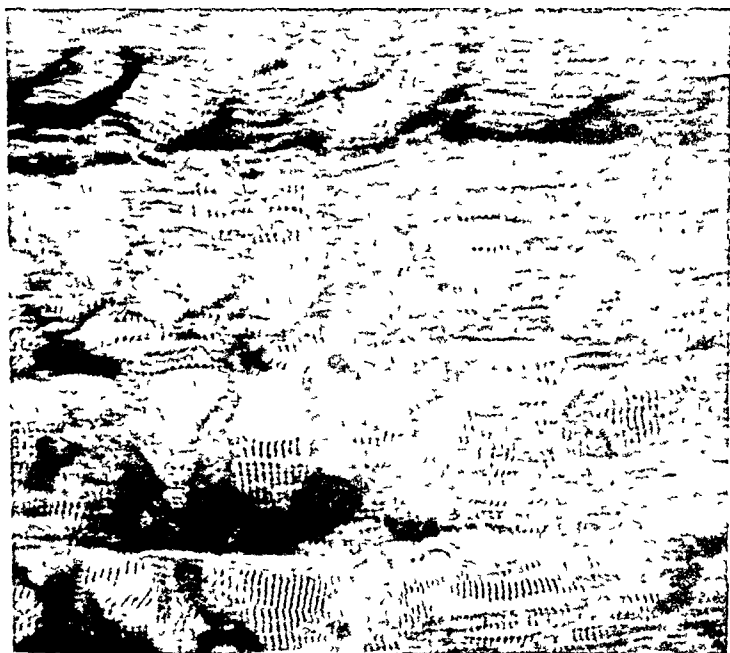


FIG 250 —Striated muscle of gastrocnemius of frog fixed in cold Bouin's fluid while tetanizing. H and E  $\times 600$  (From Meneely, courtesy of Anat Rec.)

Nerve endings are numerous and of two main types with minor variations. Conspicuous among the sensory ones are the muscle spindles. These are complicated structures consisting of one (Fig 251) or more small muscle fibers, closely associated motor fibers, sensory fibers frequently wrapped as spirals about them, plus the necessary blood vessels, the whole encased in a connective tissue capsule all its own. Many photomicrographs of sections of muscle spindles are given by Tower (1932).

The motor fibers lose their myelin sheathes and spread out in arborizations (motor-end plates) closely applied to the sarcolemma. Some are shown in figure 248. Carey (1942b) has been active in their investigation and has demonstrated changes in shape in different conditions.

Blood supply is rich and amounts to 1400 to 4000 capillaries per cubic mm which however is much less than that in cardiac muscle (Krogh, 1932). It is possible that not all are conducting at one time so that here also the usual physiological reserve is present. The plugging of a single arterial twig seldom brings about necrosis as in those tissues supplied by end-arteries.

Interesting is the silence about the lymphatics of striated muscle for their absence would be as important in a constructive effort to understand the situation as their presence. Drinker and Yofsey (1941) cite a single author, who claims to

have demonstrated them in abundance, and go on to say however that statements in the literature indicate that these vessels run in fascial planes in and also in the muscles and further that the muscular contractions squeeze interstitial fluid (its fluid) out into them for removal. The well developed lymphatic drainage of cardiac muscle is in sharp contrast and suggests a difference in the fluid environment of these two types of muscle.

We can picture therefore strands of skeletal muscle as made up of many large elongated multinuclear cells containing highly viscid contractile material enclosed in remarkably stout cell membranes well innervated and vascularized but rather lacking in lymphatics. These cells once developed must serve a long time because new ones are not ordinarily formed to take the place of those which wear out although when injured the nuclei may multiply with the production of new contractile material (regeneration) within the limits of the cell membrane. However, after some injuries the development of new fibers has been reported (Lorbus 1929).

Skeletal muscle cells are very responsive to alterations in their living conditions and must have incentive to work and materials. When the nerve supply is interrupted skeletal muscle cells very promptly become depressed. There is a decrease in size (atrophy) in which even the cells within the muscle spindles have (Tower 1932). The factors operating are of great importance in connection with paralyzed muscles in victims of poliomyelitis. Experimental studies by Hines (1942) on rats brought to light a prompt decrease in weight, strength and creatine content of muscle after denervation. Recovery quickly follows reinnervation but this is retarded by immobilization of the muscles—a kind of treatment which is consequently of doubtful value. On de-

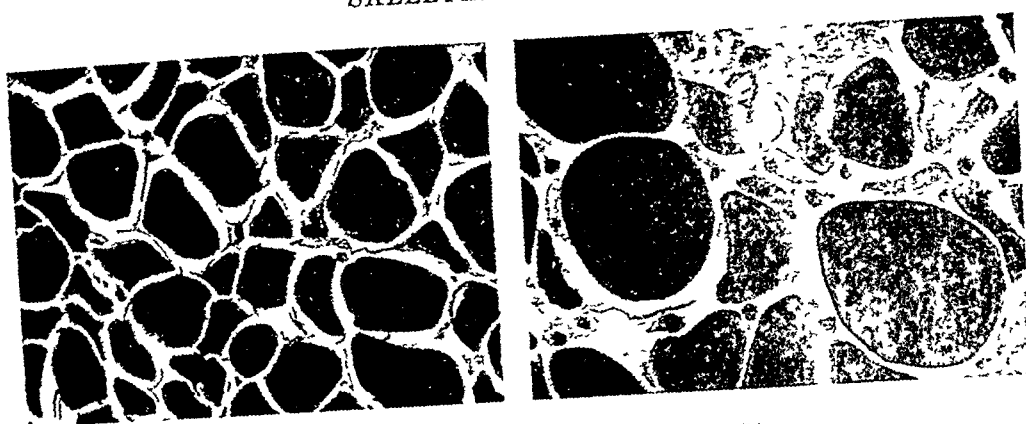
innervation there is also a rapid fall in glycogen content (Lazere *et al* 1943). Work on this problem is so intensive that other changes will probably soon be demonstrated.

On the material side there have been at least two unexpected discoveries. Androgenic hormone brings about an hypertrophy of skeletal muscle and an improvement in work performance. This is discussed by Todd and Fale (see Cowdry 1942). Vitamin I deficiency causes in animals, and probably in man, severe injury to skeletal muscle which smooth muscle apparently escapes (Lagereheuer 1942 1943). Other chemical needs of skeletal muscle are of course less known.

Some age changes in the cells have been worked out histologically by Bercu.

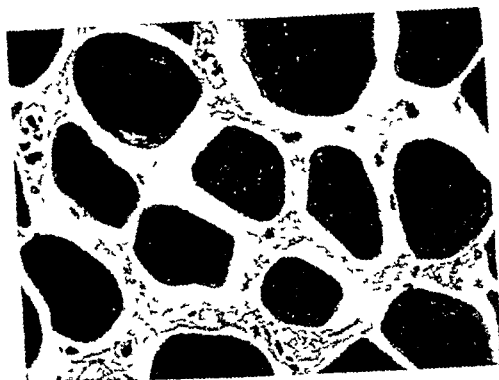


FIG. 251 —Innervation of a muscle spindle in the intercostal muscle of a hedgehog. Methylene blue preparation. (Hoche in Penfield's Neurology. Paul B. Hoeber Inc.)

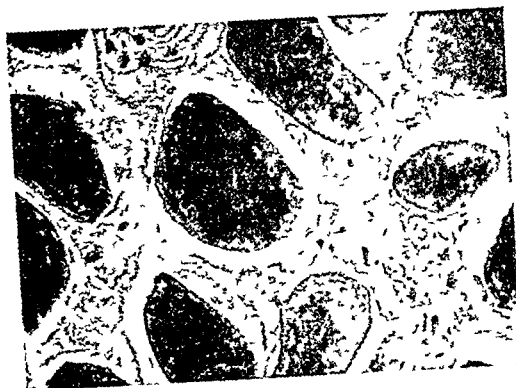


24 years

51 years



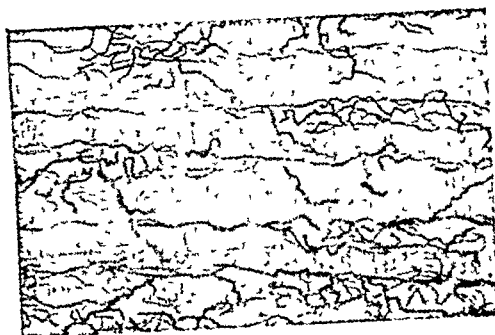
73 years



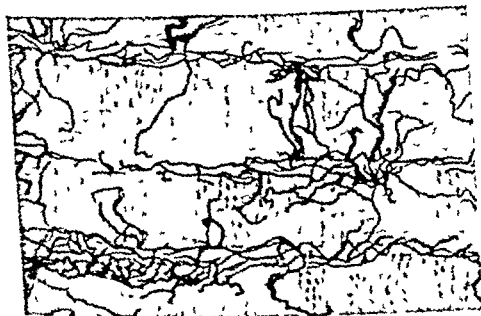
81 years



6 years



22 years



65 years



91 years

B

FIG. 252 —A, Increase in size of fibers and in volume of intermuscular tissue with ageing in sternocleidomastoid muscle.  $\times 360$  B, Increase in elastic tissue (shown in black) with ageing of superior rectus muscle  $\times 600$  (Redrawn and modified from Bucciante and Luria, Arch. Ital. di Anat. e di Embriol.)



and Iuria (1934) The number decreases but size increases also volume of muscular tissue and the amount of elastic tissue (Fig. 212)

Lowry and Hastings (1942) have made a histochemical study of alterations in rat skeletal muscle with age In the entire interval of from thirty to one thousand and ten days—the equivalent of the span between three and one hundred years in man—there was little change in the water content except for a slight rise in the oldest group Instead of the dehydration claimed in extreme age these authors think that there is a hydration which may be occasioned by an extracellular edema consequent upon atrophy or loss of cells or perhaps result from cardiac or renal hypofunction Actual evidence for change in composition of the cytoplasm that remains functionally active in very old individuals is in their opinion still lacking It would however be unwise to let up in the search for such changes

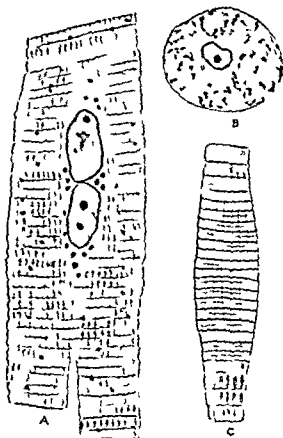


FIG. 2a3—Cardiac muscle of sheep. A Longitudinal section. B transverse section. C contraction band as seen in longitudinal section.  $\times 800$

**Cardiac Muscle**—As already pointed out (p. 76) the heart is essentially a blood vessel which has become a pump. The demand for more efficient contractile material than vascular smooth muscle has been met by the development of striations almost exactly the same as those in skeletal muscle. That smooth muscle of the dog's bladder when repeatedly distended with concentrated boric acid becomes striated has apparently been proved by Carey (1921b) for the first time. It unquestionably shows striated muscle although this change has not been repeated by other workers. The significance of the fact that in cardiac muscle the striated contractile material is accumulated about centrally placed nuclei is obvious.

skeletal muscle it displaces the nuclei to the periphery of the cell, and even flattens them against the cell membrane, escapes us. Cardiac muscle cells are obviously much smaller than skeletal ones. They can be seen to branch and the branches to extend roughly parallel to the cell body (Fig 253). The width of the cells is evident but their ends are so ill-defined that it has been said that they form a syncytium. Certainly the cross-striated fibrils,

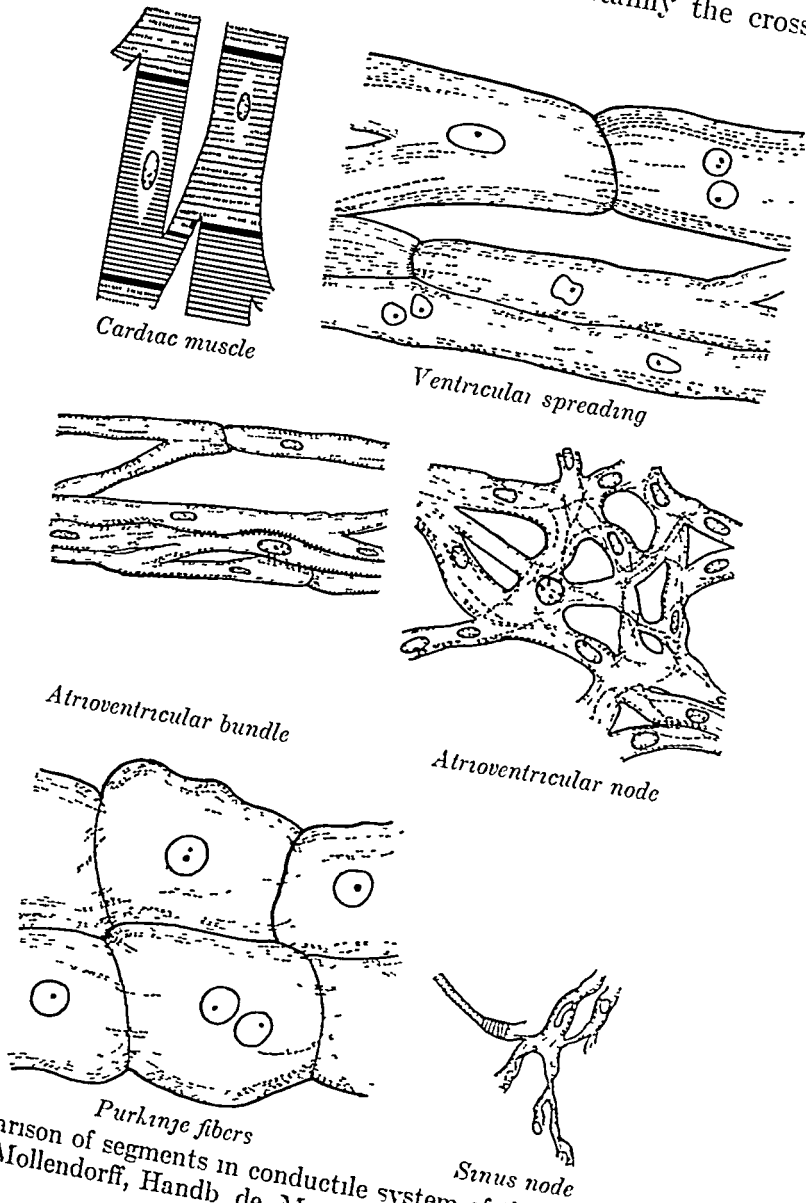


FIG 254 — Comparison of segments in conductile system of the heart (Redrawn from Benninghoff, in Von Mollendorff, Handb de Micr Anat des Menschen, courtesy of Julius Springer)

viewed after fixation, are continuous end to end from the region of each nucleus, or clump of nuclei, to the next. But there exist, at what are believed to be the surfaces of contact between cells in series, peculiar differentiations, known as *intercalated discs* which are absent in smooth muscle and almost invariably lacking in skeletal muscle though they have been described and illustrated in some skeletal muscles by Carey (1936b). In ordinary preparations these discs exhibit a different affinity for stains than the other parts of the fiber. They color either more intensely or less

intensely. In figure 253, 1 they appear as light bands stretching across the fiber at right angles. One is shown above and two below the point of branching. It looks as if in their formation one row of dark bands is suppressed. Sometimes the intercalated disc does not extend all the way across the fiber at one level but passes a little way, stops abruptly, and continues one or more segments higher. This may be repeated so that the disc assumes a step-like appearance. There are two reasons for supposing that the discs mark the ends of cells. The first is that at the point there is often (but by no means always) a conspicuous change in the degree of relaxation or contraction of the fiber. The second is that in the pathological ex-

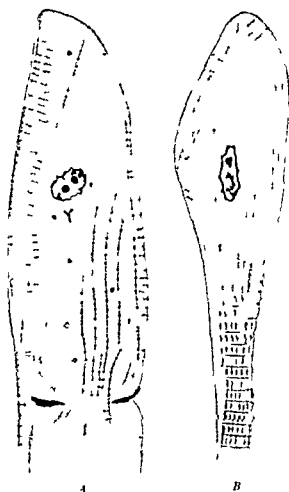


FIG. 253.—Purkinje cells of sheep. A shows part of intercalated disc on either side of it. B transition between a large Purkinje cell and a smaller cardiac muscle cell.  $\times 400$ .

dition of myocardial segmentation the fibers break up into segments (or cells) at the discs. Saphir and Karsner (1923-24) have shown by ingenious experiments that segmentation depends upon two factors: the condition of the intercalated discs and the tension upon the fibers during dilatation. Their photomicrographs should be consulted.

Cardiac muscle cells must be serviced so that a sufficient number of them can function a long time. To this end they have a richer blood supply than skeletal muscle and drainage of tissue fluid is supplemented by lymphatics (p. 78). They have no need of muscle spindles but nervous regulation is fortified by a rich

conduction system made up of modified muscle the arrangement of which has been discussed (p. 80). In fact muscle exists in the heart in many forms as illustrated in figure 254. The myocardium is a privileged tissue possessed of many inherent protective mechanisms (p. 81). Age changes show themselves in several ways including a thinning of the cells and an accumulation in them of lipofuscin (the wear and tear pigment) in the condition spoken of as brown atrophy. It is questionable whether the cells ever divide and produce new ones, though this has been described (Warthin, 1924)

**Purkinje Muscle.**—These cells deserve special mention in our comparison of contractile cells. Like the cardiac muscle cells they are elongated bodies with blunt ends separated each from the other by intercalated discs. The best place to find them in sections is just beneath the endocardium. Purkinje cells are larger than cardiac muscle cells with which they may be continuous (Fig. 255). Their pattern of architecture is the same with central nuclei and peripherally placed striated myofibrils but the proportions of the components are different. The nucleocytoplasmic ratio is less and there is relatively much more non-contractile undifferentiated cytoplasm. The functional interpretation of these strange cells is one of the unsolved problems. It is said that they can increase in number by longitudinal fission (Todd, 1932).

#### SUMMARY

All muscles are of mesodermal origin with the single exception of the iris muscles which are ectodermal. All muscle cells serve by contraction. They learn to do this in the various tissue fluids in which they grow up. The service rendered is adjusted to local needs for instance in the muscular tissue of the intestine, in the biceps muscle, in the myocardium and the Purkinje fibers. Since these are of four principal kinds we find four types of muscles easily recognizable by their structure. In no other system is the opportunity to relate form and function more enticing. This is because contraction is a function fairly easily measured quantitatively and qualitatively. There are already numerous structural details to be associated therewith, and the way is rapidly being prepared for the inclusion of traits far beyond the limits of microscopic vision such as the character and the arrangement of molecules. Not only are structure and function to be contrasted but also the responsiveness of cells of the four types to alterations favorable and unfavorable in their tissue fluid environments. For proper performance they must maintain a fairly even keel. Atrophy, hypertrophy, regeneration and tumor formation are among the most obvious of these responses in which marked differences present themselves for correlation. As the years fly by some muscle cells fall by the wayside and others age. The muscular system is ever but a part of the whole organism and it may fail partly in consequence of what is happening in other parts of the body especially in the nervous system where the number of cells capable of activating muscle decreases.

## CHAPTER XVIII

### MALE REPRODUCTIVE SYSTEM

This consists of principal and accessory parts. The first are the sperm and hormone producers the testes and the second comprise the excretory ducts through which the sperms are discharged and the glands (seminal vesicles prostate and bulbourethral) which supply the necessary fluid medium. Unless students familiarize themselves with the gross anatomy of this system the following account will be difficult to understand. In it particular attention is not given to the intricate details of spermatogenesis which once related are soon forgotten but rather to easily observable structural features and their significance.

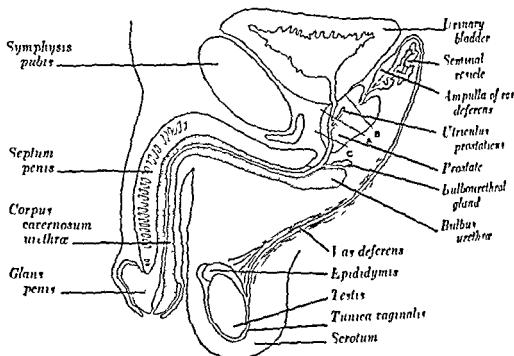


FIG. 276 - Diagram of median section of male sexual organs. A transverse section through the prostate at A is represented in figure 271. (Redrawn and modified from Eberth *Bardleben's Handb. d. Anat. d. Menschen*.)

**Testis Architecture** - Owing to its size the testis is seldom viewed in complete transverse or longitudinal sections. Often but small pieces are presented for examination which require to be oriented in terms of the whole organ. In doing so the diagram presented in figure 277 may be helpful.

Usually a little of the capsule is included in the section. This is seen to be a thick investment made up chiefly of white (or collagenic) connective tissue with few blood vessels which in the living condition is white hence the name *tunica albuginea* (*albus* white). Its external surface is smooth and covered

with a thin visceral layer of slippery mesothelium (seldom found in sections) to permit freedom of movement against the opposed parietal layer of mesothelium

The anterior border and lateral surfaces of the organ, as well as both extremities, are enclosed in a serous cavity comparable to the peritoneal sac.

The substance of the testis consists of vascularized loose connective tissue, plus interstitial cells (p 324), and many tubules which in sections are cut at almost all angles and seem to be without orderly arrangement. In the diagram, the localization of these tubules in 9 lobules is indicated. The lobules are in reality much more numerous and closely pressed together. They have thin investments of connective tissue, converge to the posterior part of the testis, and are most evident in transverse sections parallel to their length at their tapering bases where the tubules straighten out (*vasa rectæ*). Figure 258 illustrates this transition and a clip

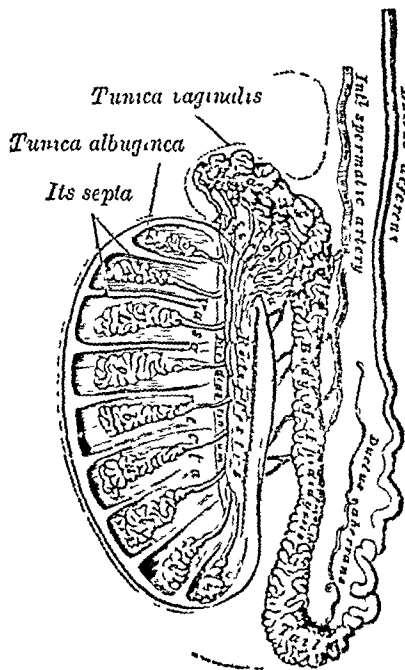


FIG. 257.—Vertical section of the testis, to show the arrangement of the ducts. (Gray's Anatomy)

through the connective tissue boundary of a lobule. In the most posterior part of the testis, obviously not immediately under the capsule, the straight tubules merge and form a network (*rete testis*, Fig. 259). Here there is no mesothelial investment, this is the attached base of the organ where the blood vessels and nerves enter.

The *seminiferous tubules* constitute the structural units. These, like the urinary tubules, have been isolated by maceration and examined as individuals. They form peculiar arches, without parallel elsewhere in the body, with both ends discharging into the straight tubules and the bodies reaching out into the lobules (Fig. 260). The number of tubules in the human testis does not appear to have been accurately counted. Statements in the literature place the number in each testis somewhere between 400 and 600 and in each lobule between 1 and 4. The total length end to end of all the tubules in the human testis has been estimated to be 800 feet by Johnson (1934).

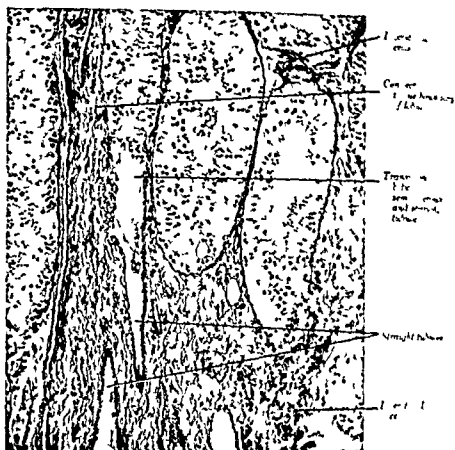


FIG. 258 Transition between testis tubule and straight tubule. Also connective tissue partition between lobules. Same specimen as figure 261. About  $\times 10$ .



FIG. 259 Same specimen as figure 252.  $\times 120$ .

**Spermatogenesis.**—This process always takes place from within outward, that is to say from the basement membranes of the tubules toward the lumina. It is easy to recognize the principal cell types (Fig 261)

1 Spermatogonia (*G sperma*, seed + *gonē*, generation) are sperm generating cells. They make up the vast majority of the cells in touch with the basement membrane. They are to be distinguished from the Sertoli cells by their rounded nuclei, rich in chromatin, and their close application to the membrane. When spermatogonia multiply the resulting daughter cells require more space than the original cells. Some are displaced in the direction of least resistance toward the lumen, while others remain in the same environment as their progenitors. The

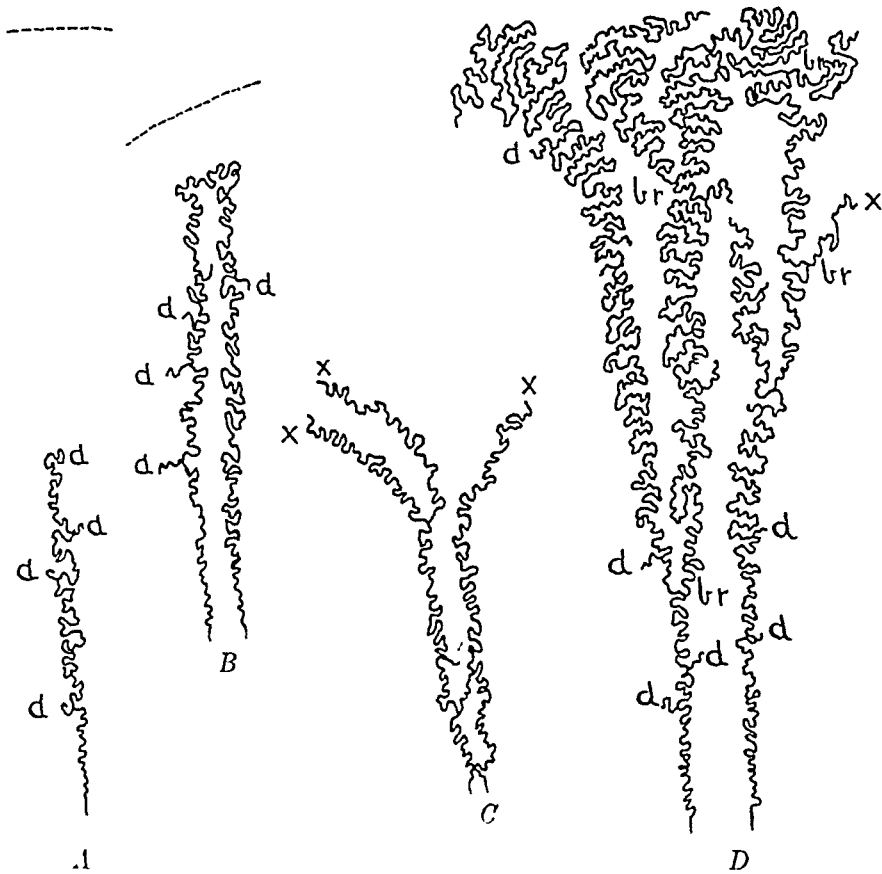


FIG 260—Seminiferous tubules of different types. A, blind end, B, simple loop, C, anastomatic branching, and D, two adjacent tubules joined by simple loop. br, branching; d, diverticulum ending blindly and x, tubule broken and not followed further. Actual size (Redrawn from Johnson, courtesy of Anat. Rec.)

former begin to differentiate, the latter, by repeating the process, start others on the path of specialization and maintain a reservoir of new cells nearest to the blood supply next the basement membrane. For many years generation after generation of spermatogonia in unbroken lines serve in the same basic capacity as other vegetative intermitotics which produce blood and epidermal cells.

2 Primary spermatocytes are produced by differentiation of the displaced daughter cells of the spermatogonia. Owing to the large size of their nuclei and the frequency of mitosis, they are the most conspicuous cells in the series.

3 Secondary spermatocytes are smaller and have smaller nuclei. They are more numerous and nearer to the lumen.



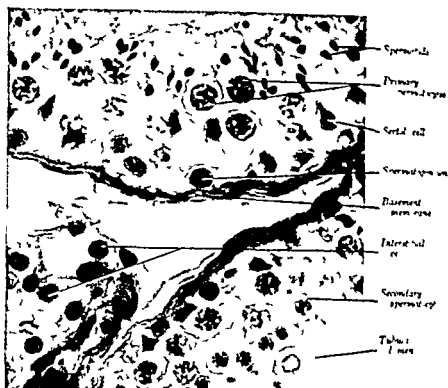


FIG. 261 - Spermatogenesis in right testis of man aged fifty-four years, castrated for prostatic carcinoma. H and E.  $\times 125$ . (Lent by Dr Earl T. Ingle)

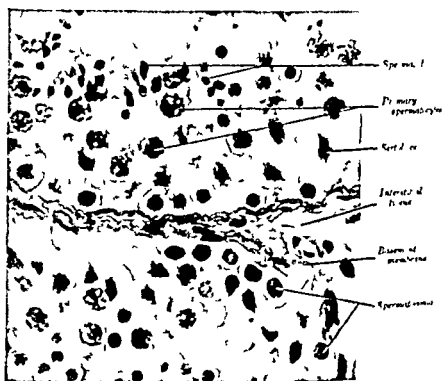


FIG. 262 - Spermatogenesis in right testis of man aged eighty-six years, castrated for prostatic carcinoma. H and E.  $\times 625$ . (Lent by Dr Earl T. Ingle)

4 Spermatids, produced in turn by division of the secondary spermatocytes, are still smaller cells. Studied in routine specimens they are readily identified by their very intensely stained nuclei, which are small, elongated bodies disposed in clumps.

5. Sertoli cells are tall pillar-like structures which extend from the basement membrane to the lumen. The converging clumps of spermatid nuclei point to their distal parts in which they are in fact imbedded. Their lateral borders are likewise indistinct being excavated by pressure from the roughly spherical spermatocytes. Their nuclei often show some lateral compression with long axes vertical to the basement membrane. The appearance of these nuclei is distinctive. They have less affinity for basic dyes than any others in the tubular wall and are marked by

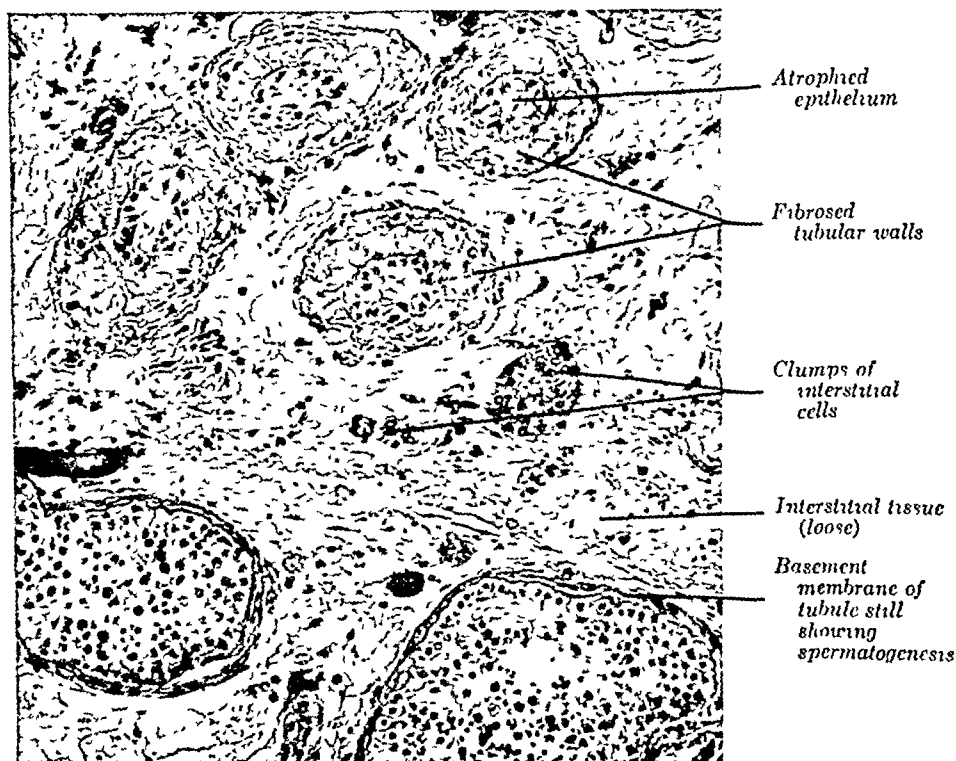


FIG 263 — Advanced and medium tubular fibrosis and interstitial cells in right testis of man (No 138) aged seventy-seven years, dying post-operatively in excision of benign hypertrophied prostate. H and E.  $\times 120$  (Lent by Dr Earl T. Engle.)

single, large nucleoli. Though Sertoli cells are not themselves differentiating intermitotics in the spermatogenic series, they play an essential and remarkable rôle in maturation of the spermatids. They seem to nurse the spermatids within their distal cytoplasm by supplying them with essentials which they get from their roots at the basement membrane and which otherwise would have to reach the spermatids in the tissue fluid seeping between layers of closely packed spermatogonia and spermatocytes.

6. Sperms are the final post mitotics developed from the spermatids and shed into the lumen tails first. They are best seen in the semen (p. 336).

During spermatogenesis the chromosomes are reduced in number to half of the 48 present in somatic cells. On union of the sperm with the egg the original number is restored. But all chromosomes are not alike. The sperms are of two sorts con-

tuning respectively 23 ordinary chromosomes plus 1 X chromosome and 2 ordinary chromosomes plus 1 Y chromosome, whereas all the eggs have 23 ordinary chromosomes plus 1 X chromosome

Sperm (23 + X) + egg (23 + X) = a female (46 + 2 X)

Sperm (23 + Y) + egg (23 + X) = a male (46 + X + Y)

Details are to be found in papers by Punter (1924) Evans and Sney (1925) and in Wilson's book (1925)

Examination of almost any testis shows marked regional variability in sperm production. This is noted when the section passes longitudinally through a tubule and is not uncommon even in single cross-sections of a tubule. It is likely that in humans as well as in mice and rabbits (Curtis 1918) the process passes like a wave along the tubule. We need more light on the conditions that lead to a per-

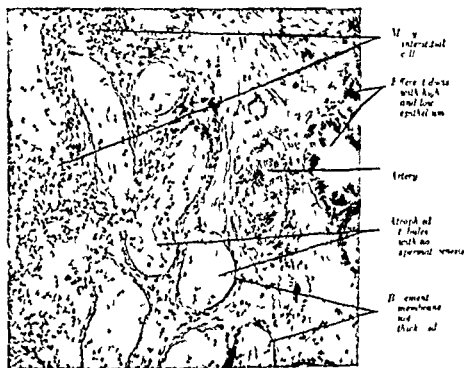


FIG. 264. Cryptorchid testicle. No spermatogenesis. Interstitial cells abundant.  $\times 170$

sistence of spermatogenesis in some individuals (Fig. 262) and its partial cessation in others who are younger (Fig. 263). Endocrine regulation is a fact and its features are becoming clear. Breakdown with advanced age is described by Ingle (1942).

Sperm production like blood cell production and probably in many other activities is sharply conditioned by temperature. It suddenly dawned upon people in Scotland, Japan and the United States at about the same time and independently (Crew 1922, Fukui 1923 and Moore and Quek 1923) that this is why the testes are held in the scrotum, virtually outside the body, where the temperature is 1 to 2° lower than in the abdominal cavity. For a long time previously it had been common knowledge that abdominally retained (cryptorchid) testes do not produce sperm. The appearance of such a testis is illustrated in figure 264. It will be seen that spermatogenesis is uniformly lacking and that most of the epithelial cells resemble those of Sertoli. Figure 265 shows stages in experimental cryptorchidism and figure 266 the regeneration of spermatogenesis in a testis re-placed in the

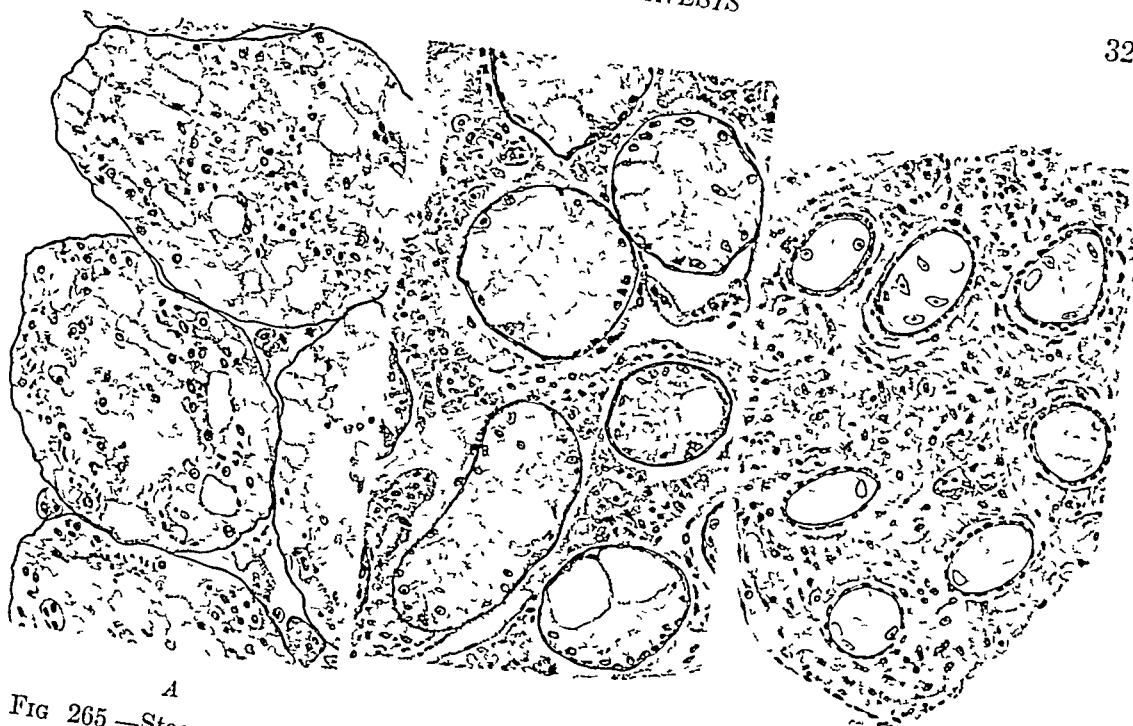


FIG 265—Stages in experimental cryptorchidism produced by confining the testes of guinea-pigs to the abdominal cavity for *A*, seven days, *B*, three months, *C*, six months. Note the progressive degeneration of the tubules and the increase in interstitial cells (Redrawn and modified from Moore, Allen's Sex and Internal Secretions, Williams & Wilkins Company)

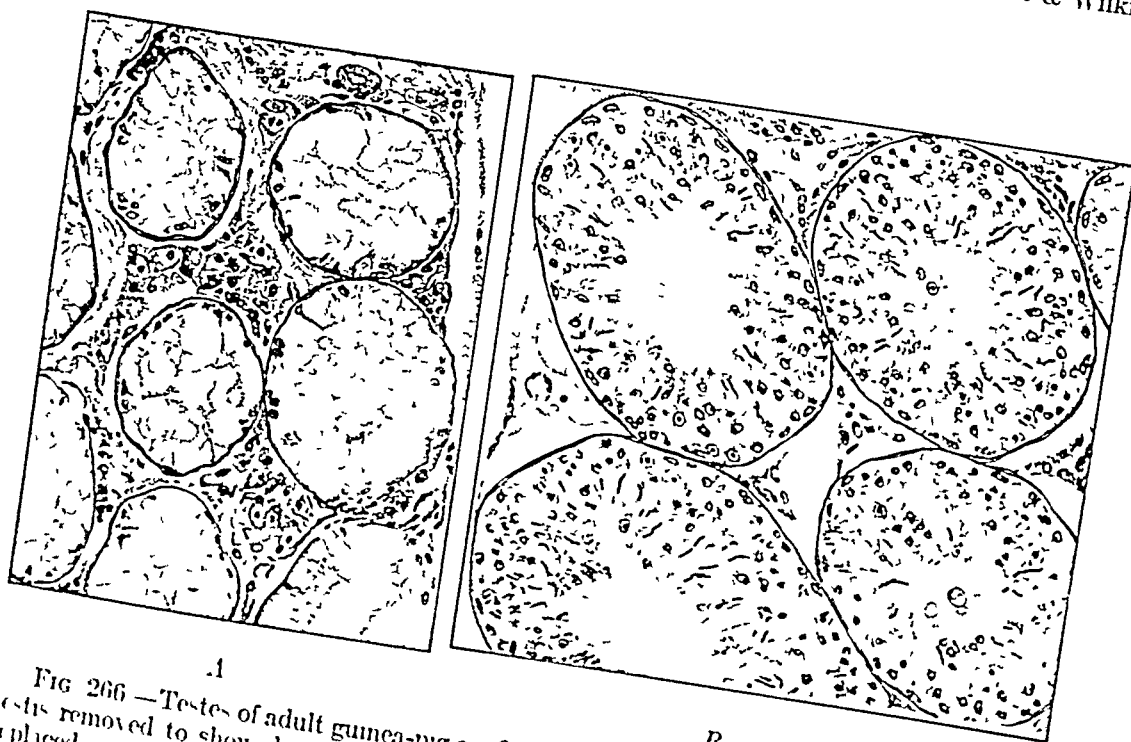


FIG 266—Testes of adult guinea-pig confined to abdomen for twenty-four days *A*, Left testis removed to show degenerate condition of both testes at this time. *B*, Right testis replaced in scrotum for period of seventy-four days. Complete spermatogenetic recovery in many tubules (Redrawn and modified from Moore, Allen's Sex and Internal Secretions, Williams & Wilkins Company)

scrotum in which degeneration owing to confinement in the abdomen had progressed too far

**Sex Hormone Terminology** — This is only mentioned here because the structure of the male and female reproductive systems is very largely hormone regulated and it is quite impossible to obtain a clear first view of structural changes as long as vague ideas persist concerning the names of sex hormones

Most of the confusion results from proprietary terms introduced by manufacturers who wish to market their products under attractive names About 100 of these are listed by Pratt (Allen's Sex and Internal Secretions 1939) The number will continue to increase See Hormones in New and Nonofficial Remedies published yearly by the American Medical Association

But there is no longer any excuse for loose usage of scientific terms Indignant meetings of distinguished committees in Europe under the League of Nations and in this country have led to agreements which have been crystallized in recommendations by the Council on Pharmacy and Chemistry (J A M A 1936 106 198 1809, 107, 200-212 1221-1223) Briefly interpreted these provide for 3 classes of sex hormones and for some individual hormones

**Androgens** (*G anēr* man + *gennaō* I produce) are principally concerned in the development and maintenance of the male secondary sexual organs and of secondary sexual characters such as masculine hair growth voice and psychological traits All substances possessing masculinizing activity are androgens In this class the Council recognizes three chemically pure hormones androsterone dehydroandrosterone and testosterone of which the latter is most used

**Estrogens** (*G oistros* made desire) are the female counterparts of the androgens They are concerned with the female secondary sex organs and characters They produce estrus and are feminizing Estrus is the recurring condition of heat or sexual receptivity in most mammals It corresponds to the menstrual cycle in women If knowledge of these hormones had first been secured by studies on humans instead of on lower animals they might as well have been designated menstrogens Three chemically pure hormones are recognized estrone estradiol and estradiol with accepted synonyms theelin thecol and dihydrotheelin respectively

**Progesterone** is the progestation hormone It prepares the uterus for implantation of the ovum and has no androgenic or estrogenic properties It is commonly called the corpus luteum hormone to signify its origin and luteosterone from *luteum* yellow *sterol* a class of chemical substances with the suffix *one* indicating a ketone By some it is known as corporin **Progestin** is a kind of generic term employed for substances having similar action produced in the placenta and found in extracts of the adrenal cortex

**Hormone Production** — Between the tubules is loose connective tissue carrying blood vessels nerves and lymphatics The usual connective tissue cells are present but fat cells are for some reason extremely rare Certainly they would be an inconvenience if present in large numbers

The designation interstitial cells is reserved for still other cells between the tubules which are hormone producers and are represented in figures 258 261 263 and 264 They may occur singly but are generally encountered in rather dense clumps When this is the case there is a faint resemblance to epithelial cells that has prompted use of the adjective epithelioid which is however quite unjustified Typically each cell has a single spherical nucleus and a considerable amount of cytoplasm charged with a good deal of fats and lipoids Crystalloids are not a few

quently found in the cytoplasm which may also contain pigment. Interstitial cells are of mesenchymatous origin and some of them look like fibroblasts which have rounded up and have been otherwise changed. A helpful review of their properties throughout the vertebrate scale is given by Rasmussen (1932).

Until recently evidence of hormone production has rested on the weak presence and absence argument. The trouble is that quantitative measurements of interstitial cells are difficult to make. The cells may occur abundantly in some parts of the testis and be almost lacking in others. A false impression of increase in number may result when the seminiferous tubules are atrophied and occupy less room in consequence of which more interstitial cells may be found in an area of the same extent in the absence of any absolute increase. The reverse also holds, that with enlargement of the tubules the number of interstitial cells may seem to be less.

It is true, however, (1) that the interstitial cells persist in tubular atrophic abdominally retained testes (Figs. 264, 265) which continue hormone production (2) that descended testes showing advanced tubular atrophy may exhibit numerous interstitial cells coupled likewise with hormone production (Fig. 263); and (3) that interstitial cell tumors may be associated with increased discharge of hormone. But on the association, if any, between reduction in interstitial cells, and decrease in hormone production, more data are required.

More convincing are the observations, reviewed by Engle (1942), that the interstitial cells are themselves hormone regulated and a part of the system. Particularly significant observations have been reported by Pollock (1942) that steroid compounds, with the chemical properties of testosterone, are confined exclusively to the interstitial cells and are not detectable elsewhere in the testis. While these cells constitute the principal source of this and perhaps of other physiologically active substances, loosely called *androgens*, and *testis hormones*, it is unlikely that they are the only source, for similar substances are to be found in females and in castrated males. Adrenal cortical cells may be involved (p. 125). The broadcasted chemical messages have a profound influence not only on the development of secondary sexual features and on maintenance of secretory activity by the accessory glands but also on skeletal muscle, renal and other tissues.

We conclude this brief outline of the histology of the testis by a tabular comparison of the behavior of its two principal tissues.

BEHAVIOR OF INTERSTITIAL CELLS AND TUBULAR EPITHELIUM

Condition	Interstitial cells	Tubular epithelium
Cryptorchidism (abdominally retained testicles)	Unmodified or more prominent	Greatly degenerated
Increase in temperature	Unmodified	Rapidly degenerate
Decrease in temperature	Unmodified	Rapidly degenerate
Vasectomy	Unmodified or more prominent	Degeneration
Röntgen-ray	More resistant	Less resistant (except Sertoli cells)
Radium	Unmodified or increased	Spermatogenesis completely arrested
Vitamin E deficiency	Proliferation	Degeneration
Denervation	Unmodified or increased	Degeneration

Let us now trace the course of the sperms from the tubules to the semen discharged in ejaculates.

**Tubuli Recti, Rete Testis, Ductuli Efferentes** — When first cast off by the Sertoli or nurse cells sperms are young and probably not motile. They still possess a noticeable amount of cytoplasm. They find themselves crowded in a thin aqueous medium which is most likely a kind of transudate from the tissue fluid surrounding the tubules. For there is no acceptable evidence that either the spermatogenic or Sertoli cells are secretory. But the possibility that the latter may discharge some sort of material or materials should not be entirely lost sight of. Their cytoplasm is more extensive and more diversified in respect to granules, crystals and other microscopically visible components than that of the spermatogenic cells. How the sperms are passed from the intermediate part of the coiled and convoluted but loop-like seminiferous tubule to the straight tubules is a question that has not been answered. The walls of the tubules do not possess muscle, the contractions of which could propel them. Some investigators invoke a kind of growth pressure, the sperms being pushed onward by more and more new ones, so that they leave by the only possible exits, since the tubules cannot dilate very much on account of the strong capsule surrounding the organ. This may be supplemented by hydrostatic pressure. It is conceivable that the epithelial walls of the seminiferous tubules are so constructed that water is filtered through, as in the renal glomeruli, but much more slowly. The spermatogenic waves which travel along the tubules are characterized by accelerated cell division. This can be taken to signify an increase in fluid and in materials of hematogenous origin, so that each wave may be accompanied by a gradual washing or flushing-out of the tubule. The waves as described by Curtis are, however, not as regular as we would wish to justify this explanation, and they seem to pass from the beginning of the straight tubules into the testis instead of along the seminiferous tubules toward the straight tubules.

The outside diameter of the short straight tubules (*tubuli recti*) by which the sperms and fluid pass directly from the seminiferous tubules to the rete is narrower (Fig. 235). If the combined cross-sectional area of their lumina is less than that of the seminiferous tubules opening into them, the current through them will be faster. They are lined by simple columnar cells said to be akin to Sertoli cells and containing like them a good deal of fat and lipid.

On entering the *rete testis* (Fig. 239) the sperms find themselves in a network of wide irregular spaces where there can be but little onward current. The spaces are lined by cuboidal and even by squamous epithelium. The rete is really a complex system of connecting lakes of microscopic dimensions. The strong fibrous basement membrane of the seminiferous tubules is reduced in the straight tubules and wholly absent in the rete.

The upper posterior part of the rete is drained by a series of 15 or more efferent ductules (*ductuli efferentes*) which spread like the leaves of a fan to join the ductus epididymidis. These ductules are much coiled and contorted. They are more highly differentiated than the straight tubules or the rete. It is in them that the first definitely secretory cells are found as well as the only cells provided with motile cilia. The former are often grouped together and are shorter, so that they constitute little pockets (intra-epithelial glands) or grooves in the ciliated wall (Fig. 264). The nature of the secretion has not been determined, but it is known that the ciliary beat is in general onward toward the epididymis (details given by Zawisch-Oczutz 1933). The efferent ductules are also supported by a basement

membrane and provided with a delicate circular layer of smooth muscle, the contraction of which pushes on the sperms and fluid

**Epididymis**—The long, convoluted *ductus epididymidis* receives all of the efferent ductules and acts as a storehouse for the sperms. Its wider and more circular lumen is lined by a pseudostratified, secretory, columnar epithelium. The projecting, distal, brush-like ends of the cells are fibrillated and have been termed stereocilia, but they are not motile. It is in this duct that the sperms mature. Young (1931) has discovered that the sperms removed from the distal end (next the ductus deferens) are much more effective as fertilizing agents than those collected from the proximal part (near the entry of the efferent ductules). The secretions of the seminal vesicles, prostate and bulbourethral glands (Cowper's glands), added later to the sperms, are non-essential. Toothill and Young (1931) have

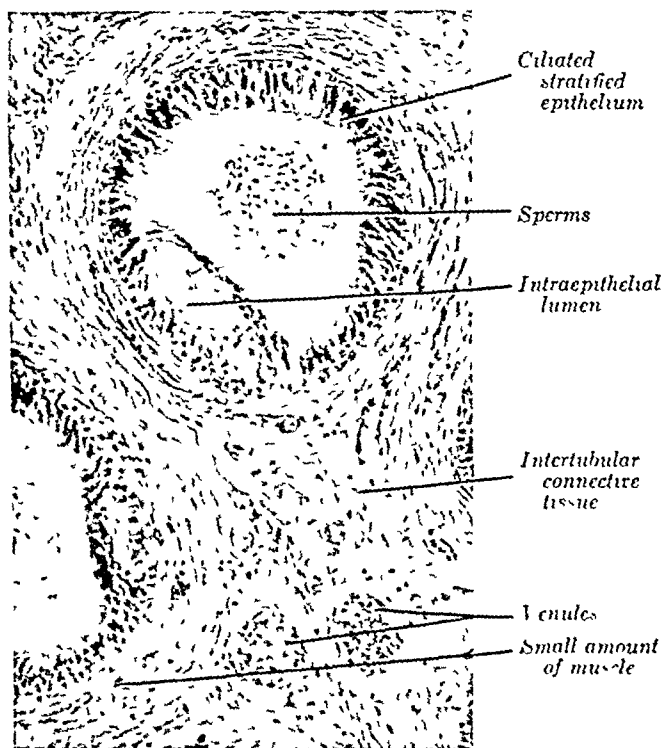


FIG 267—Epididymis, human. Formahn-Zenker. H and E

observed that particles of India ink, injected into the proximal part of the guinea-pig's epididymis, require about fifteen days to make the journey to the ductus deferens. The wall of the epididymis is equipped with basement membrane and some circularly disposed smooth muscle fibers (Fig 267).

**Vas Deferens.**—Sperms arriving at the *ductus (vas) deferens* die unless quickly forced out in ejaculations. Each ductus on nearing the prostate expands into a kind of ampulla, receives the secretion of a seminal vesicle, traverses the prostate as an ejaculatory duct and empties into the intraprostatic part of the urethra (Fig 256). It is a long tube lined by a pseudostratified columnar epithelium which in fixed preparations is thrown into folds due, perhaps, to contraction of the walls. In proportion to the diameter of its lumen the ductus deferens is one of the most heavily muscled tubes in the body. Its firm, wire-like consistency, felt through the thin skin of the upper part of the scrotum, is unmistakable. The muscles are



of the smooth variety and arranged in three layers—inner longitudinal muscle, circular and outer longitudinal. It would be worthwhile to ascertain whether they are exactly circular and longitudinal or have an inclination to spiral disposition as in the alimentary tract. The same layers, less developed, make up the wall of the ureter, but the foldings of the epithelium of the undilated ureter are much deeper and more regularly slit like and the epithelium, by contrast, is of the transitional

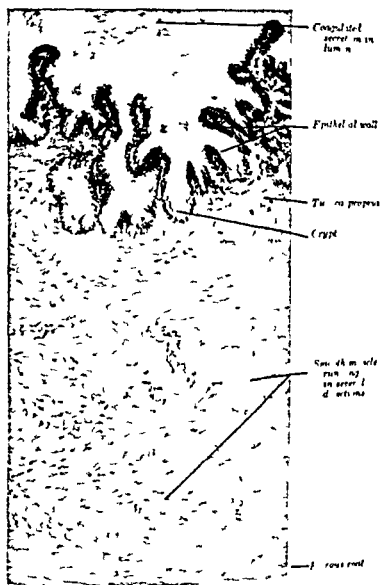


FIG. 208. Vesicula seminalis human. Formalin-Zucker. H and E.

stratified variety made up of four or five cellular strata, the innermost of which (facing the lumen) is composed of large, bloated-looking cells. The ductus deferens is conductile and the large amount of muscle is indicative of the degree to which the sperms and fluid in it can be evacuated. It is not appreciably secretory. For its proper maintenance it depends, like the other accessory male reproductive organs, upon a continuous supply of testis hormone. When this is lacking the ductus deferens atrophies.

**Vesiculæ Seminales.**—The seminal vesicles are to be regarded as appendages of the excretory ducts of the testes. In some animals they are lacking. Each vesicle is a very irregular and contorted tube which joins the ductus deferens at the point where the latter changes into an ejaculatory duct which pierces the prostate and discharges into the prostatic urethra (Fig 256). When freed from its capsule this tube is about 10 cm. in length. According to McCarthy, Ritter and Klemperer (1927), its capacity is from 2 to 7 cc. Figure 268 shows the structure of the vesicular wall. The physiological histology of the organ is critically reviewed by C. C. and M. T. Macklin (1932). The seminal vesicles are not simply storehouses for semen as the name implies. The extent to which they act as resting places for the sperms is questioned and they may not serve in this capacity at all (see Beams and King, 1933b). They are certainly secretory in function. The product may be recognized in the ejaculated semen by its yellow color and thick, sticky consistency. The

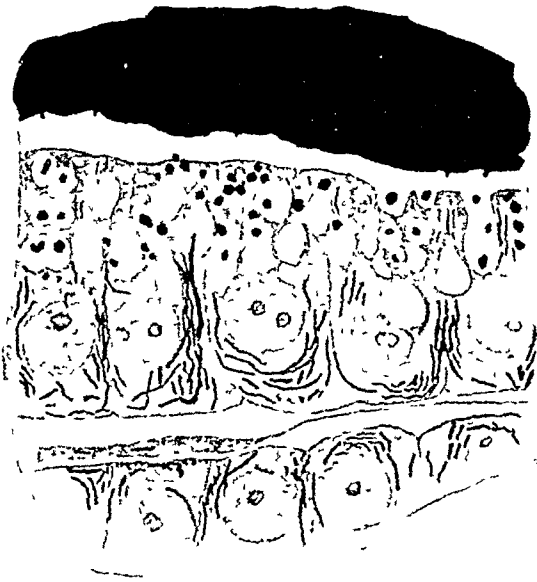


FIG 269 —Secretory process in vesicula seminalis of mouse from a specimen fixed in a mixture of formalin and potassium bichromate and stained with fuchsin and methyl green. The tips of some filamentous mitochondria seem to project into watery vacuoles. Spherules of secretion antecedent are seen and a mass of secretion is present in the lumen.  $\times 1500$  (Cowdry, Contrib to Embryol)

secretory epithelium accumulates a lot of lipochrome, beginning at about twenty years of age. It is much folded and exhibits many irregular diverticula. The cells are highly variable both as to length and the presence of secretory antecedents. The mitochondria have been studied in white mice. They actually project into the clear vacuoles that encase the secretory antecedents (Fig 269). Moore, Hughes and Gallagher (1930) have made the important discovery that the cells respond to testis hormone to a degree that can be measured quantitatively. Their illustrations should be consulted. They show that in some cases the epithelial cells of the normal rat's vesicula seminalis are columnar bodies about six times as long as they are wide, containing many granular secretion antecedents in their distal cytoplasm. When the influence of the hormone is removed by extirpation of the testes, the secretion antecedents disappear and the length of the cells gradually decreases until they have become cuboidal. The injection of testis extract into a castrated

animal promptly restores the length of the cells and their secretion anteriorly. The epithelium lies on a vascularized connective tissue supported by muscle which is made up of interlacing strands and ill-defined circular and longitudinal fibers. The response of this muscle to the female sex hormone theelin has been investi-



FIG. 270 — Showing influence of theelin on seminal vesicle of the rat. *A* Section of vesicle removed from a rat, aged six months, which was castrated when two months old. *B* Section of the second vesicle later removed from the same rat after receiving 15 days of subcutaneous injections each of 20 rat units of theelin. Note the marked growth of muscle and the absence of change in the epithelium.  $\times 1$ . (Overl. for a 1X lens.)

gated by Overholser and Nelson (1934). Further experiments are described by Wells (1936). They have discovered likewise in the rat that theelin injections bring about a tremendous increase in muscle and exercise a depressing effect on epithelium (Fig. 270). This observation raises many interesting questions and

ing the mechanism of muscular hypertrophy in the pregnant uterus. There are many non-myelinated fibers and a few nerve cells can be made out. An excellent discussion of innervation of the entire reproductive tract is that of Gruber (1933).

**Prostate.**—The position of the prostate is illustrated in figure 256. It stands before the bladder (Gr. *prostates*, one standing before) and is a meeting place of the urinary and male genital tracts. The best account of the histophysiology of the prostate is given by C. C. and M. T. Macklin (1932). Ageing is discussed by Moore (1942).

In incomplete sections which have not been made in a known direction this gland is a bewildering mass of secretory tubules, follicles, connective tissue, smooth muscle, blood vessels, nerves and lymphatics. The section under examination must first be oriented. If it passes as indicated by the line *A* in figure 256, one may expect to find some of the structures shown in figure 271 which is highly schematic.

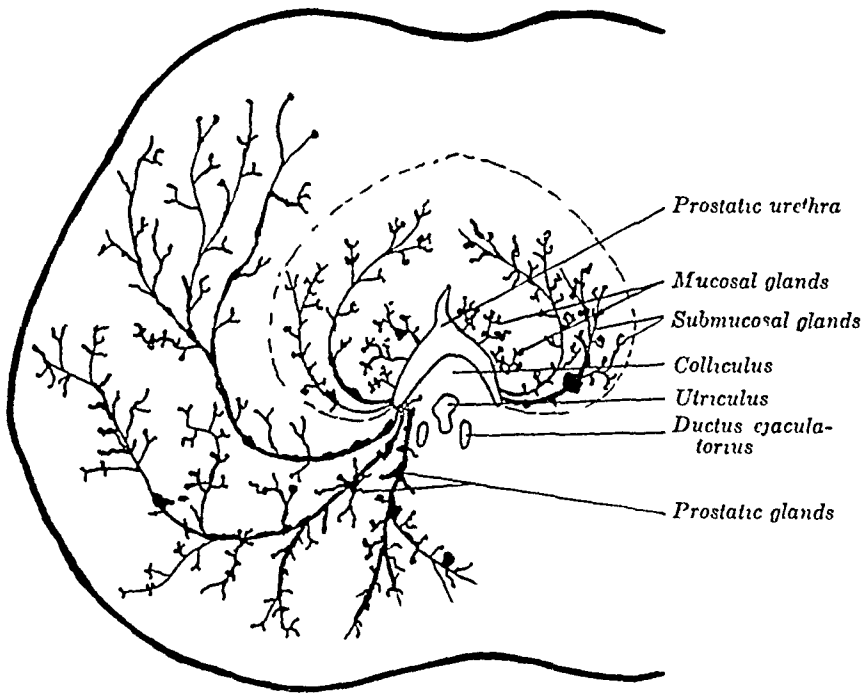


FIG 271 —Diagram of cross-section of prostate in the plane indicated by the line *A* in figure 256

The urethra, utriculus and ejaculatory ducts are represented in the dilated state although they are seldom so found in sections owing to the escape of fluid from the organ and the contraction of the smooth muscle fibers about them. The shape of such a transverse section is very much like one through the mid-brain. Its anterior surface facing the symphysis pubis is slightly concave, and its posterior surface, which can readily be palpated through the rectum, is more so. The outline of the lumen of the prostatic urethra, near where the semen enters, is distinctive. In figure 271 it is represented as a moderately dilated, triradiate space. The floor always projects inward as a little hill (*colliculus*, L. dim. of *collis*, hill). The crest of this hill, also called the verumontanum, points forward. In the mid-line beneath this hillock is the *utriculus*—a blind pouch that approaches the urethra at about the same angle as the ejaculatory ducts (Fig. 256). The function of the utriculus is obscure. It does not serve as a duct for the prostatic glands but its epithelial

lining may contribute a small amount of secretion. The utriculus opens at the summit of the colliculus. The ejaculatory ducts are paired tubes situated below and posterior to the utriculus. They make their way into the colliculus and discharge by slit like apertures into the urethra. If the section is made at *A* ventral to the urethra nor the utriculus will be seen but the two ejaculatory ducts will be present. If it is lower at *C*, it will contain the urethra with just a trace of appreciable colliculus and without utriculus or ejaculatory ducts. Obviously sections through the side of the prostate wholly devoid of these landmarks are very difficult to orient.



FIG. 272.—Prostate of sixteen year old male. Fixed in 10 per cent formalin and stained with H and E.  $\times 140$ . (Dept. Pathology, Washington University, No. 10086, lent by Dr. R. A. Moore.)

The glands occur in 3 groups—submucosal, mucosal and prostatic. As indicated in figure 271 the last named are much the largest. Though originally tubular they change in adults into a series of communicating follicle-like spaces (Fig. 272) at first slightly reminiscent of the isolated ductless follicles of the thyroid (compare Figs. 272 and 101). Both are typically lined by a single layer of epithelium which may be columnar, cubical or squamous. Storage of secretory products is a marked feature of both but the epithelium and the products are more diversified in the prostate. Large laminated prostatic concretions which may become calcified are very characteristic and the tissue between the follicles in the prostate is largely smooth muscle which is absent in the thyroid. Contraction of the muscle facilitates exit of secretion which is a thin alkaline fluid responsible for the peculiar odor of semen. It is not yellowish like the secretion of the vesicles but is slightly of a bluish color. Note that sections of lactating mammary glands (Figs. 203 and 204) can also look



FIG 273 —Prostate of rat as testis hormone indicator A, Normal, B, twenty days after castration, C, twenty days after castration but receiving 29 injections of testis extract in the twenty days (Moore, Price and Gallagher, Am J Anat)

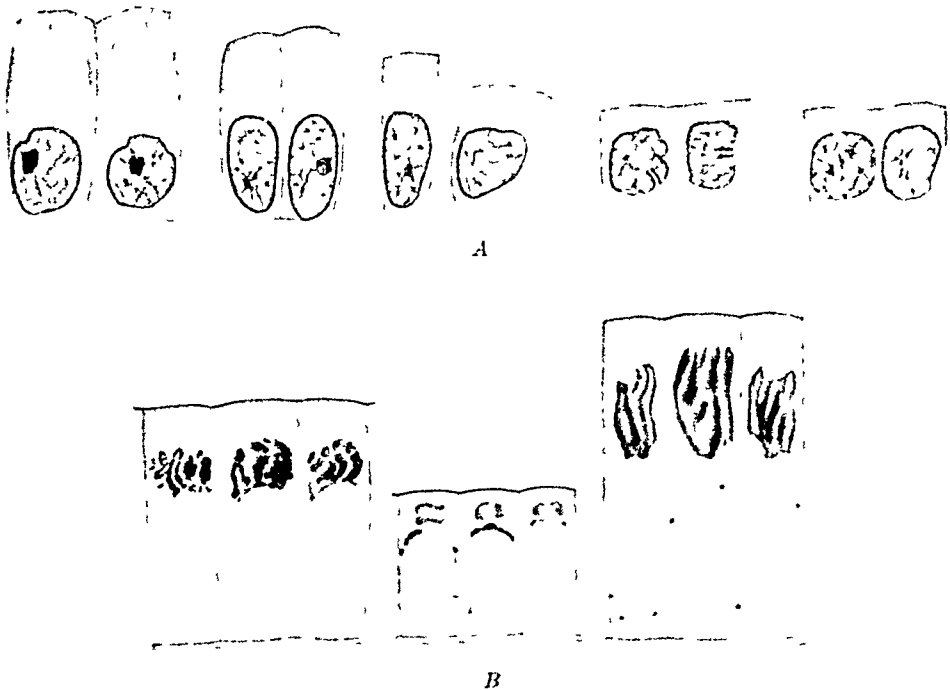


FIG 274 —Middle lobe of prostate gland of rat used as testis hormone indicator. A, Development of castration changes from normal (left), four-day castrate, ten-day, twenty-day and ninety-day castrate (right) B, Showing Golgi body reactions— Left, normal, center, twenty-day castrate, right, twenty-day castrate injected with testis extract (Moore, Price and Gallagher, Am J. Anat)

faintly like those of a young prostate but the contents of the lumina are directly fatty, as revealed by clear spaces from which the fat has been dissolved and no muscle is absent.

Moore Price and Gallagher (1930) have observed that when by castration the sustaining influence of the testis hormone is eliminated the prostate atrophies but that it soon recovers on the administration of testis extract (Fig 273). They have in addition measured the degree of reaction by determining the alterations in the height and structure of the secretory epithelium (Fig 274). In view of this remarkable dependence of the normal prostate on testis hormone it is not surprising that the cancerous prostate as well as metastases therefrom should also undergo atrophy in 75 per cent of patients treated by castration (Huggins *et al* 1941).

The prostate is almost unique among glands in its tendency to undergo benign hypertrophy in advanced years. This enlargement is statistically normal because Moore (1942) gives its incidence at well over 60 per cent in individuals over sixty years of age. According to Moore Miller and McLellan (1940) older men suffering from this condition show in general a great reduction in urinary excretion of androgens (male hormones) than do others of similar age without enlargement. If this means a reduction in amount of androgens in the circulation their prostates are certainly not reacting by atrophy to decrease in hormone as in the rats investigated by Moore Price and Gallagher and in prostatic cancer.

**Bulbourethral Glands**—These only require passing notice. Their location is indicated in figure 256. They may be larger or almost vestigial and form a mucous lubricant secretion. Between the secretory tubules and alveoli there is a good deal of muscle part of which may be striated. They are also called Cowper's glands. The urethra has been considered with the urinary system. It has other small discharging glands (of Littre).

**Penis**—From the histological point of view reference is only necessary to its remarkable blood supply which serves not only to nourish the tissues but to cause erection in response to psychogenic and other stimuli. The process of erection has been studied by Kiss (1921). The branches of the arteries to the erectile tissue and of the veins which leave it are constructed differently from any others in the body. On cross-section the arteries look distinctly pathological. They are seen to be partly filled up by ridges consisting mainly of longitudinally arranged smooth muscle fibers (Fig 275). In the relaxed condition of the penis these arteries are very tortuous and this smooth muscle is considered to be in tonic contraction like the regular circular muscle. When erection begins this tone is lost and the arteries are thought to straighten out and dilate enabling the blood to rush into the erectile tissue. It is known that erection also results from artificial stimulation of the nerve endings which carry parasympathetic vasodilator fibers from the sacral cord and presumably decrease muscular tone. Some of the veins are equipped with funnel-shaped valves which retard the overflow, and the largest ones are probably also compressed by contraction of the ischio- and bulbocavernosus muscles. Consequently the organ swells. An idea of the extent to which the cavernous blood spaces of the corpora cavernosa and the corpus spongiosum are puffed up with blood may be gained by comparing A and B figure 276. Rigidity is given by the fact that these bodies are prevented from undue expansion by firm fibrous investments comparable to outer automobile tires. Following ejaculation the longitudinal and circular layers of muscle regain their tone and the inflow of blood is reduced the penis becomes flaccid. Swelling of the corpora cavernosa of the nasal mucous membrane and

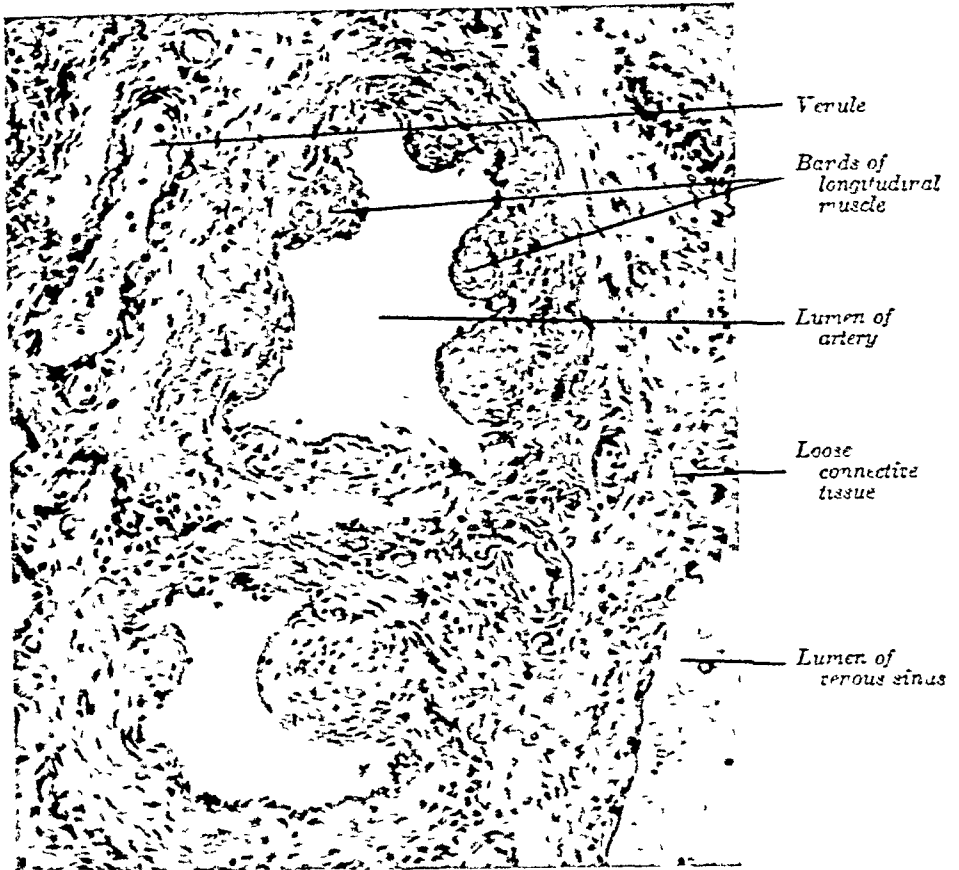


FIG 275 —Infant penis—spiral arterioles.  $\times 180$ .

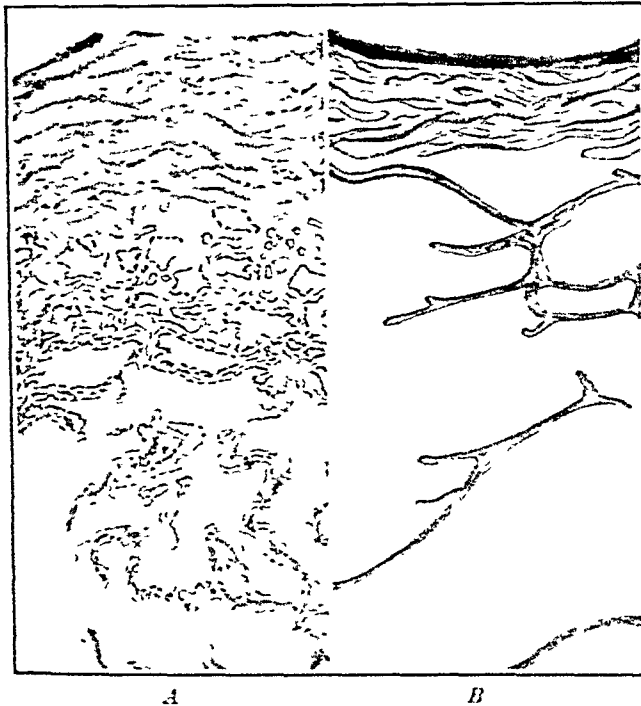


FIG 276 —A Cross-section of a crus penis of an individual aged twenty-seven years, in the relaxed state. B Cross-section in erected state to indicate vascular engorgement. (Redrawn and modified from Kiss, *Ztschr. f. Anat.*)



erection of the clitoris of females and of the nipples of males are to some extent similar phenomena

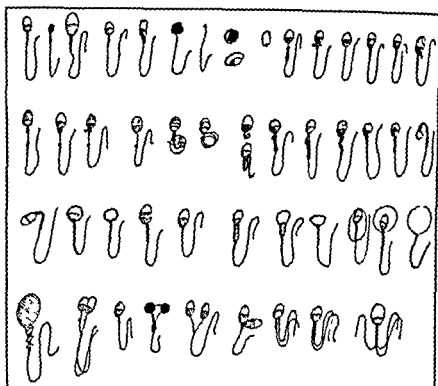


FIG. 277 — Drawings illustrating the range of variation of spermatozoa. The first is regarded as the usual form. (Redrawn from Moench and Holt, *Am J Obst and Gynec*)

**Semen** This is a complex mixture which varies with the physiological and pathological state of the testes, their ducts and appended glands. Histologically prostatic concretions, sperm crystals, lymphocytes, de-quimated epithelial cells

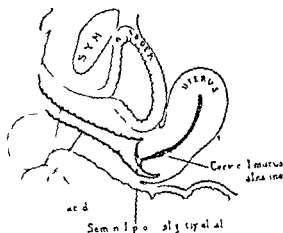


FIG. 278 — Diagram showing environment of sperm in vagina and uterus. (McClure and Kurzrok, *Am J Obst and Gynec*)

(and leucocytes when present), lipid droplets and sperm can be recovered. The latter named have been investigated from the point of view of how they may be

## SEMEN

helped (cases of sterility) and of the best way to kill them (selection of contraceptives). In structure the sperms are not all alike. Moench and Holt (1931), in particular, have studied the abnormal forms (see Fig. 277). They think that head abnormalities indicate impotency. Moench (1933) feels justified in concluding that a normal, fertile man ejaculates less than 20 per cent of abnormal sperm heads, that when the percentage rises to 20 to 25 per cent impaired fertility is to be assumed and that men producing more than 25 per cent are always sterile. This is a better measure of fertility than sperm motility, for it is now well known that ability to fertilize is often lost before motility is noticeably impaired.

Chemically the seminal fluid is feebly alkaline and since it is a buffered solution this alkalinity is protected against modification when exposed to small amounts of acid secretions. Miller and Kurzrok (1932) have made important observations on

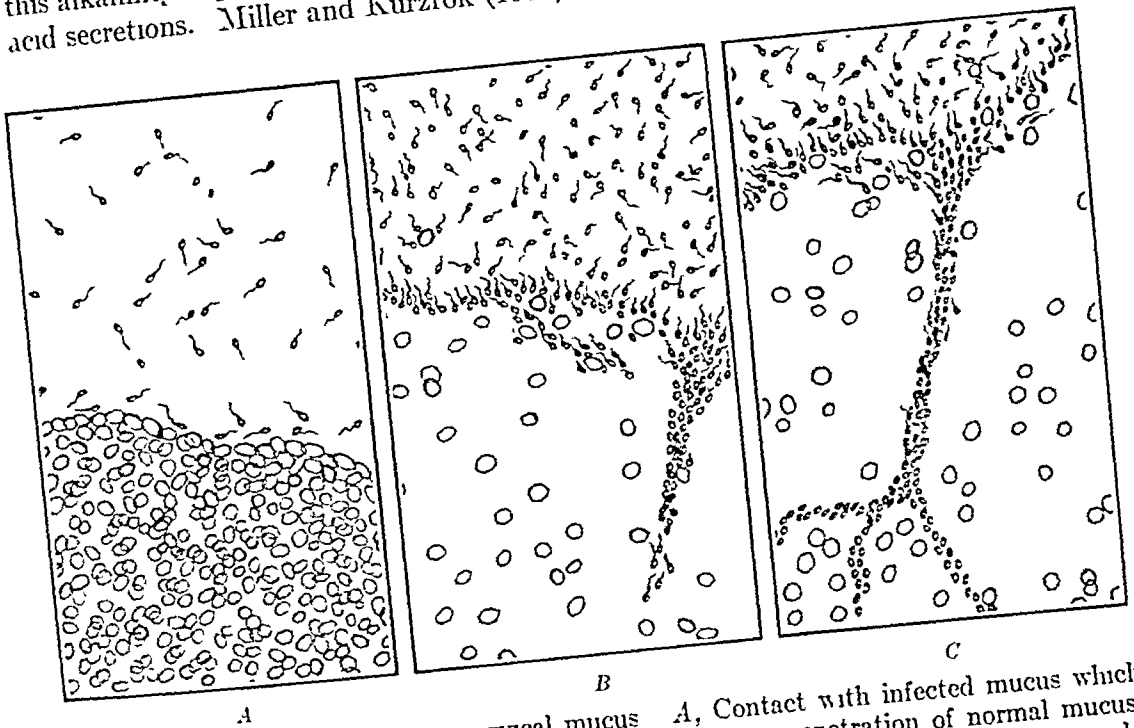


FIG 279 —Sperms attacking cervical mucus. A, Contact with infected mucus which resists penetration. B, Orientation against and beginning penetration of normal mucus. C, Further entry into normal mucus. (Redrawn and modified from Miller and Kurzrok, *Am J Obst and Gynec*)

the pH of the vaginal and uterine fluids (Fig 278) and of the ability of the sperm to penetrate mucus. In an earlier contribution they proved that the semen contains a mucolytic enzyme which acts specifically on the mucus of the normal cervix, making ready the way for the sperm. When this mucus is altered by infections the sperms are turned away as is graphically shown in figure 279. According to Killigan (1933), urea is present in the semen in approximately twice the blood concentration, sugar four to six times and lactic acid five times the amounts in the blood. As is to be expected, active sperms utilize sugar and produce lactic acid. Killigan believes that their motility increases with decrease in viscosity of the medium. The histochemistry of sperms has been investigated by Marza (1931) and their immunological reactions have been exhaustively studied (see Mudd and Mudd, 1929).

An admirable account of the discovery of sperms is given by Bremer (1936) parts of which we quote. They were first observed by a Dr Ham, whom Leeuwen-

hoek described in his communication to the Royal Society in 1677 as 'a man of singular modesty'. He wrote

This discerning youth visited me and brought with him in a small glass vial seminal fluid from a man who had cohabited with a diseased woman and he stated that after some minutes when the fluid had become so attenuate that it could be put in a slender glass tube he had seen living animalcules in it which he thought were produced by some putrefaction. He added that these animalcules seemed to him to be provided with tails and that they did not survive the space of twenty four hours. Moreover he declared that when terebinth had been given to the patient internally the animalcules appeared to be dead.



FIG. 280.—A human sperm as represented by Dalenpatus in 1699 (Redrawn from Bremer, Textbook of Histology, courtesy of P. Blakiston's Son & Co.)

"I poured this material in a glass tube and examined it in the presence of Dr. Ham and saw some live animalcules in it. But when after two or three hours I examined the material more carefully, by and by I saw that all the animalcules were dead.

Leeuwenhoek believed that the animalcules were of two sexes and that each held within it a preformed man or woman in miniature. Dalenpatus (1699) went further and claimed actually to see these tiny preformed individuals.

"We have seen some animalcules having just the form of tadpoles such as are found in brooks and muddy bogs in the month of May. The tail is four or five times as long as the body. They move with wonderful rapidity and by the strokes of their tails produce little waves in the substance in which they swim. But who would believe that in these a human body was hidden? Yet we have seen such with our own eyes. For while we were observing them attentively, a large one threw off its surrounding membrane and appeared naked showing distinctly two legs, thighs, breasts and arms. The cast-off skin drawn upward covered the head like a cap and it was a delightful and incredible sight. Because of the minuteness of the object the sex could not be distinguished. After the little creature had lost its membrane it soon died.

The impulse to see what one expects to see did not die with Dalenpatus. His illustration of a sperm is reproduced in figure 280. It looks like a complacent hooded member of the Klu Klux Klan adorned with a cross but minus a stocking.

### SUMMARY

The structure of the male reproductive system is designed for the production of sperms, their storage and introduction into the genital tract of the female in such a way that fertilization results. The life of sperms is a hazardous experience and since the chances of any particular one fertilizing an egg are almost infinitesimal they are formed in enormous numbers. Approximately 200,000,000 are liberated with each ejaculation. Their development in the convoluted tubules of the testis is fairly continuous during adult life and depends upon an adequate supply of gonadokinin from the pituitary, of vitamins especially E and of the maintenance of a temperature in the scrotum a degree or more below that of the

body cavity At the same time the testis is busy producing a hormone which serves three purposes It stimulates the development of secondary sexual characteristics It maintains the proper degree of activity of the passages by which the sperms are to leave and of the glands that afford the necessary fluids for them It also regulates testicular activity by inhibiting the production of gonadokinin by the pituitary which activates the testis The young sperms are wafted in some way from the seminiferous tubules through the straight tubules and rete into the efferent tubules by whose ciliary and peristaltic movement they are forced into the ductus epididymidis where they remain in storage for days, perhaps for months An optimum period of residence in this locality is necessary if they are to escape in a vigorous condition It has been observed that the fertilizing power of ejaculates after long abstinence and after undue frequency is low as compared with that accompanying moderate intercourse Most of the sperms that travel of their own initiative into the ductus deferens die It is only through the very definite sequence of events in ejaculation that they can escape with sufficient fluid and in large numbers The way has to be prepared and their passage expedited There is some reason to think that the fluids and sperms that make up the semen are discharged in a certain order Erection is accompanied by the exudation of a slimy lubricant from the bulbourethral glands and by contractions all along the excretory passages The alkaline secretion of the prostate follows, preparing the way by neutralizing any local acidity of the urethra Then come the sperms with the secretion of the seminal vesicles which is said to have a nutritive value Unless the fluids of the vagina and uterus are quite acid, the sperms remain active for hours or days, but their ability to fertilize is of shorter duration

## CHAPTER XIX

### FEMALE REPRODUCTIVE SYSTEM

THE reproductive system of the female has a far more complicated task to perform than that of the male. It must supply eggs favorable conditions for fertilization by sperms and a suitable environment and nutriment for the growth of the offspring up to the time of birth also milk by the mammary glands for several months thereafter. It is divisible into the principal sex organs the ovaries and various accessory parts including the Fallopian tubes uterus vagina and mammary glands. The system functions in cycles. Each stage is accurately timed and regulated by hormonal and nervous mechanisms. In no other system of the body have the advances made in the past decade been so illuminating. Concepts unquestioned for a generation have failed to withstand the impact of newly discovered facts for well controlled experiments are now the order of the day. A landmark has been established by Allen (1932) who at the request of the National Research Council sought out leading investigators and persuaded them to co-operate in preparing a statement of our present knowledge on 'Sex and Internal Secretions'. So great has been the demand for this book and so rapid have been the further advances that a new edition was published in 1939.

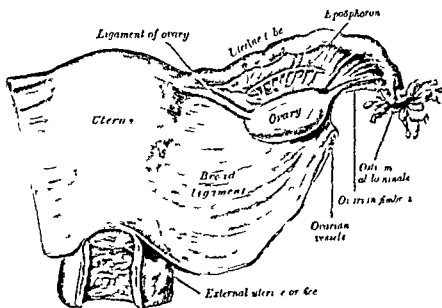


FIG. 251.—Uterus and right broad ligament seen from behind. The broad ligament has been spread out and the ovary drawn downward. (Gray's Anatomy.)

**Ovarian Architecture.** Much more than the testis the appearance of the ovary differs with age and phase in reproductive cycle. Before going into details it is desirable to recognize some landmarks. Figure 251 shows the position of the ovary in relation to the other parts of the system. Since it projects into the peritoneal

cavity most of its surface is covered with mesothelium some of which can be seen in all sections except those restricted to the substance of the organ and the attached surface. This mesothelium, commonly called epithelium, is germinal in the sense that in early life it invades the underlying tissue and produces ova. It consists of a single layer of cuboidal, or columnar, cells and is illustrated in figures 282 and 284. Sometimes this *germinal mesothelium* is brushed off in making the preparations. *Graafian follicles* constitute the second easily visible landmark. These are empty looking rounded or oval structures of large size visible to the naked eye (Fig 286). *Corpora lutea* are likewise large structures with walls much folded and made up of lipid rich cells generally oriented in irregular columns vertical to the surface strongly suggestive of the zona fasciculata in the adrenal. *Corpora albicantia* are shrunken corpora lutea so named because they look white since they are made up of connective tissue with few blood vessels so that they are essentially avascular (Fig 283).

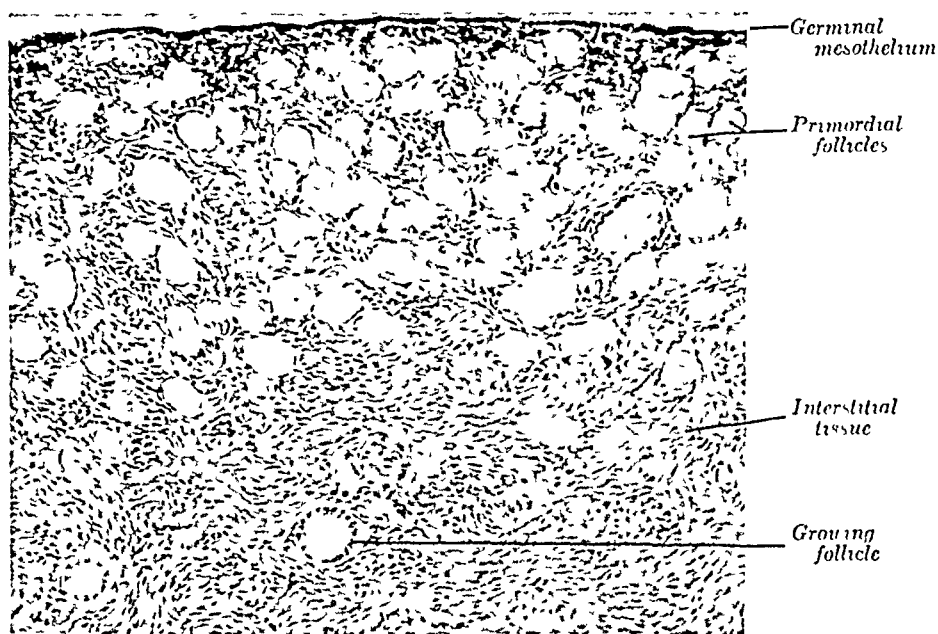


FIG 282 —Ovary one and one-half years old. Died of influenzal meningitis. Autopsy three and one-quarter hours postmortem. (Dept. Pathology, Washington University, No 10421, tissue obtained from Dr. R. E. Stowell.)

**Ovogenesis.**—The process may be arbitrarily divided into 9 stages represented diagrammatically in figure 285. (For this and many other aspects of developmental anatomy see Arey, 1940).

- 1 The germinal mesothelium has been mentioned.
- 2 From this buds project through the underlying layer of connective tissue (tunica albuginea) into the substance of the organ.
- 3 These clumps of cells are pinched off from the surface and a primordial germ cell makes its appearance as a rounded cell slightly larger than the rest. Whether this is a differentiation of the neighboring mesothelial cells, or migrates here as a primordial germ cell from the gut endoderm, remains to be determined. Everett (1913) has presented strong evidence for such a migration in mice.
- 4 The germ cell enlarges and the cells about it flatten to form a primary, or primordial, follicle.

5 This then becomes recognizable as a growing follicle by the development of two or more layers of cells about the germ cell.

6 Further differentiation is in several directions. (a) The beginning investment of the ovum by an even homogeneous eosin staining membrane produced at the surface of separation between it and the surrounding follicular cells and known as the *zona pellucida*. (b) The commencing accumulation of fluid between the follicular

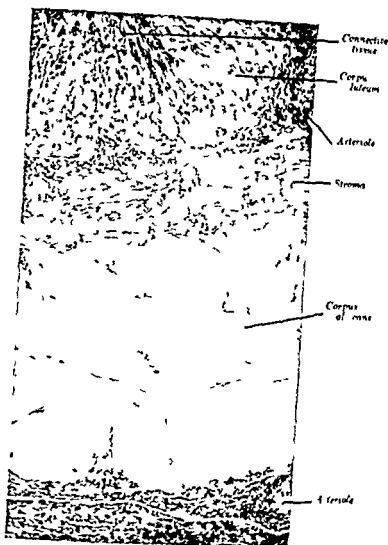


FIG. 283 - Corpus luteum (above) and corpus albicans (below) in ovary of woman aged forty three taken twenty days after last menstruation. Fixed in formalin and stained with H and E  $\times 14$ . (St. Louis Maternity Hospital Lab No 1300, lent by Dr J. E. F. Hobbs.)

cells to form the *liquor folliculi* when the follicle enlarges to the point of naked eye visibility. (c) The investment of the outer surface of the follicle by cells of mesenchymatous origin giving rise to a special covering—the *theca*.

7 Continued growth and differentiation to the *Graafian follicle* stage which is so much larger that in order to bring it into the figure it must be indicated at reduced magnification. The alterations initiated in the preceding stage are pushed to an extreme. (a) The *zona pellucida* is further developed. (b) The *liquor folliculi*

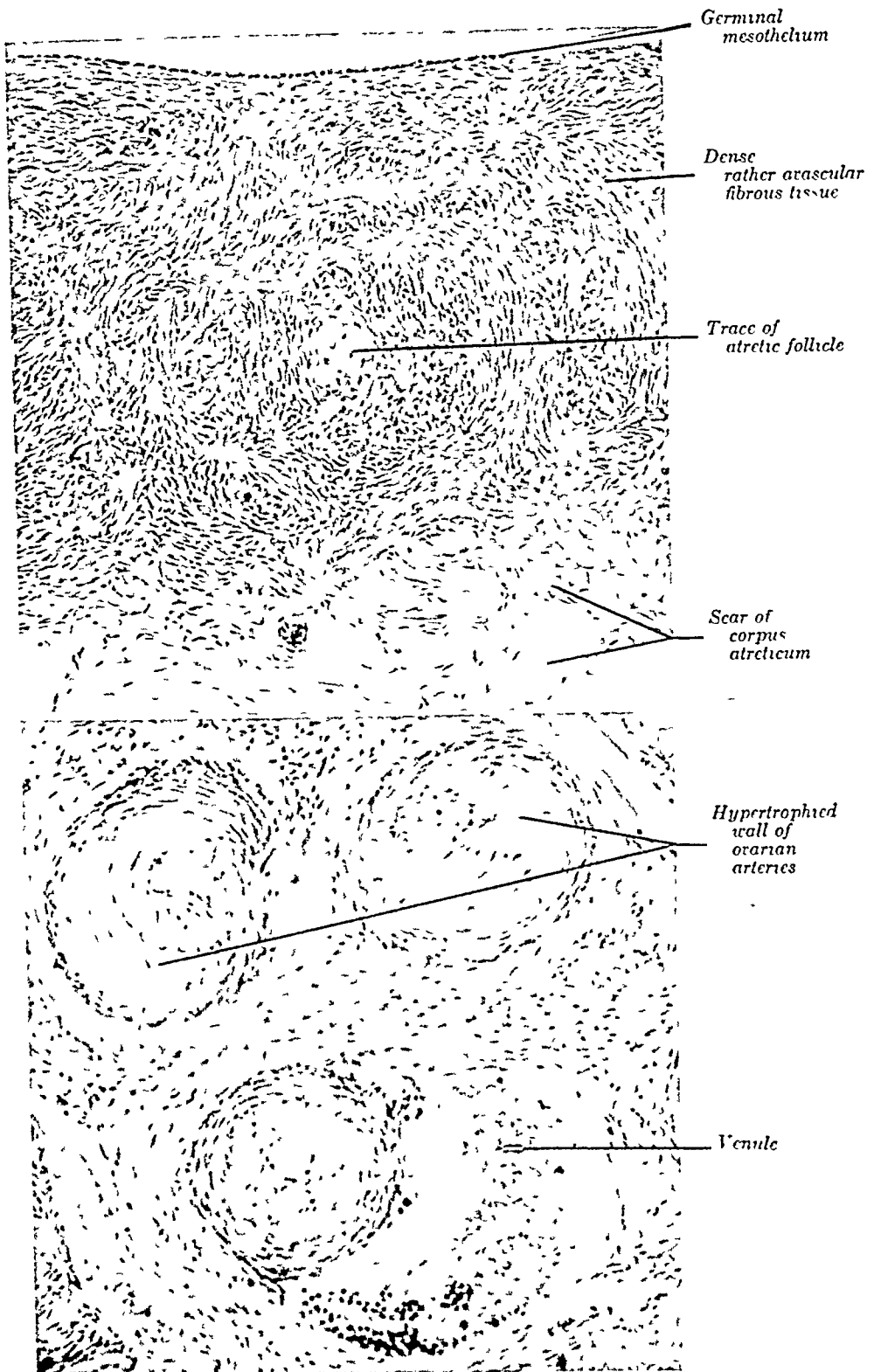


FIG. 284 —*above*, cortex of ovary and *below*, ovarian blood vessels of eighty-five year old white woman who died suddenly after being struck by a car. Autopsy one and one-quarter hours postmortem.  $\times 135$  (Dept Pathology, Washington University, No 10551, tissue obtained from Dr R E Stowell)



dominates the picture. It is limited only by a few thin layers of follicular cells—the *zona granulosa*—which are heaped up in a little hillock at one side—the *corpus albicans*, *oöphorus*—containing the ovum and by the *theca interna*. (c) The outer covering increases in thickness and forms the *theca externa* which alone is vascularized. Capillaries do not penetrate the follicle, the differentiation of which up to maturity

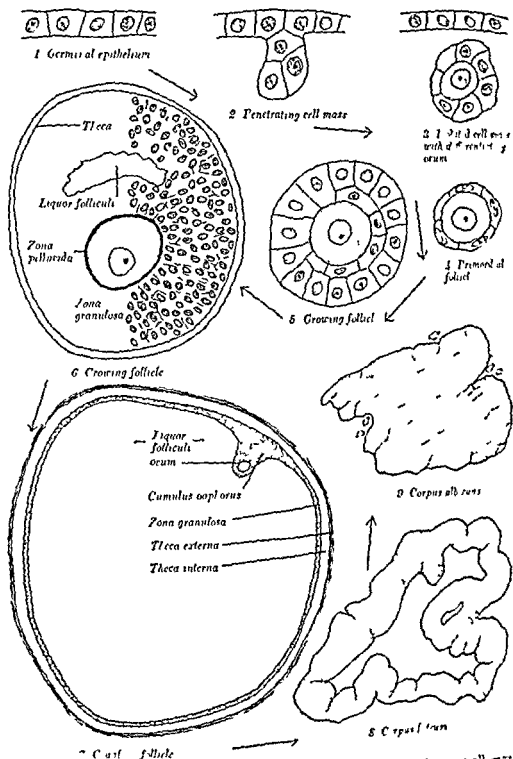


FIG. 285.—Diagram of stages in maturation of follicle and formation of corpus albicans and corpus luteum.

is dependent upon the secretory activity of the follicular cells and the fluids which slowly enter it by diffusion. The oxygen supply for the ovum, surrounded by layer upon layer of dividing and oxygen consuming follicular cells and later suspended by the cumulus oophorus in a little lake of fluid, must be relatively small so that its stored energy is conserved. While increasing in size so enormously, the Graafian follicles push aside other younger follicles and form very noticeable projections on the surface of the ovary. As the follicle enlarges, due to added secretion of follicular fluid, the cumulus oophorus with the ovum usually becomes detached from the wall by intercellular secretion at its base. The egg then floats free in the follicular cavity. The increase in fluid pressure and the physical properties of the follicular wall have been investigated by Thanhoffer (1934) by microdissection. When the follicle ruptures (ovulates) the egg, or ovum, is consequently borne out in a wave of discharged fluid. It enters the peritoneal cavity and is ordinarily picked up by the fimbriated opening of the Fallopian tube.

8 After the evacuation of its fluid contents the follicle crumples up. The granulosa becomes folded and the granulosa cells hypertrophy in cords extending radially from the periphery toward the center. At the same time the cells of the theca interna penetrate, with their associated vessels, into the mass. The subsequent changes depend upon whether the ovum is fertilized in the Fallopian tube. In the vast majority of cases this does not happen and the structure is called a *corpus luteum spurium*, or false yellow body. This reaches its greatest size approximately two weeks after the escape of the ovum, that is to say, when the next menstruation begins. At this time the structure resembles slightly the cortex of the adrenal gland on account of the arrangement of its cells in columns separated by blood vessels and a little connective tissue. The theca lutein cells are similarly laden with lipid. They have a faint yellow color due to the lipochrome pigment, lutein. Some blood may escape between the cells and into the lumen, which the connective tissue elements also enter. The red cells disintegrate and some iron-containing pigment is deposited.

9 Later on the lumen is occluded, the lutein cells degenerate and the connective tissue cells of the theca attain the ascendancy. Collagenic fibers are produced with the result that the corpus luteum shrinks, persisting only as an irregular fibrous mass possessed of but few blood vessels and therefore white in color—the *corpus albicans*. But if pregnancy supervenes the corpus luteum undergoes hypertrophy and may attain a diameter of as much as 2.5 cm. The lutein cells become larger and the yellow pigment less noticeable. After about six months this *corpus luteum rrum* regresses in much the same way as the spurium.

Only a small proportion of the follicles live to evacuate their ova and to pass through the corpus luteum stage. Estimates on the number of primary follicles in both ovaries taken together at birth range from 36,000 to 300,000 (see Schroder, 1930). Although, contrary to the older view, new follicles continue to be formed in the manner described during the reproductive life of the individual, the total number rapidly decreases. At puberty it is placed at approximately 16,000. Growing follicles and some fairly mature Graafian follicles degenerate with the primordial ones. The loss continues during the years of sexual activity. It is accelerated during pregnancy and increases with the climacteric, after which the disappearance of follicles of all sorts is complete. This process of follicular degeneration is termed atresia.

An appreciation of the extent of *follicular atresia* may be gained by vital staining

with trypan blue and similar dyes which are taken up in large quantities by the degenerating cells and the macrophages that remove them. Hundreds of ova die for every one that enters the Fallopian tube but they do not leave the scar tissue which remains from the corpora lutea and which converts the scabbed ovaries into a firm fibrous mass. The life of the individual ovum is apparently short as well as

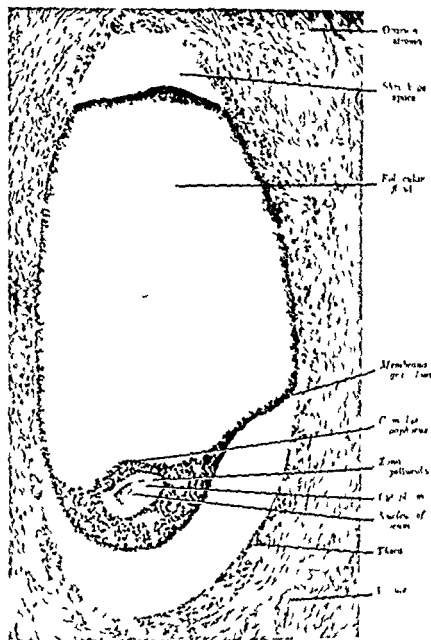


FIG. 20. Young tertiary follicle from same case as Figure 20.

hazardous. It was formerly supposed that a woman is endowed at birth with a certain number of ova and that those which do not die persist for say forty years awaiting the call to service. Now the germinal epithelium is recognized as a source for new ova and Evans and Swarz (1931) have stated that we cannot be sure whether or not we except the elements of the blastocysts do not have

the shortest life span of any cells in the body." But their evidence is from species with large litters. Data on primates is needed.

Ova, like sperms, undergo a process of maturation. Those in the primordial follicles are generally considered to be primary oocytes and homologous to primary spermatocytes. They represent the culmination of a period of multiplication and growth. The first maturation division, by which the number of chromosomes is halved, occurs in the Graafian follicle. One of the daughter cells forms a small *polar body* and degenerates. The second division begins in the follicle, halts in mid-phase, and is completed only after ovulation and subsequent fertilization. Again one of the daughter cells succumbs as a polar body (see figures 286 and 287).

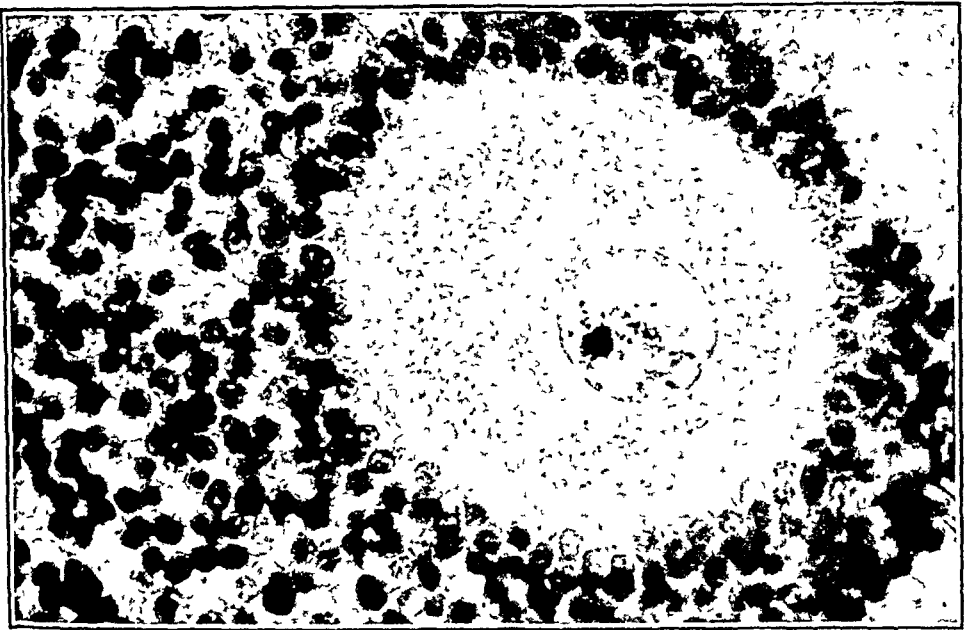


FIG 287 —Mature ovum. Carnegie collection. (Gray's Anatomy.)

These changes cannot be followed in the usual class material. Therefore it is worthwhile to view two fine moving picture films which can be obtained on loan from the Wistar Institute.

1. Early development of rabbit egg *in vitro* (long film) by W. H. Lewis and P. W. Gregory.
2. Early divisions, 2 to 8 cells, of living monkey egg *in vitro* by W. H. Lewis and C. G. Hartman.

The *interfollicular tissue* of the ovary includes blood vessels, nerves and lymphatics as well as smooth muscle fibers and connective tissue. The smooth muscle is not disposed in sheets or bundles but pervades the organ. A few fibers encircle the follicles. Shrinkage, on muscular contraction, may facilitate the discharge of the distended Graafian follicles. The connective tissue elements are interesting and important. As already indicated the corpora albicantia are simply masses of collagenic fibers. Phagocytes are always active in association with follicular atresia. Fibroblasts are numerous but here as elsewhere the dividing line between them and mesenchymatous cells is indefinite. One or the other or both contribute to the formation of the follicular theca. In addition certain large cells with spherical nuclei and much lipid have been described under the heading of *interstitial cells*, or epithelioid cells. They resemble the interstitial cells of the testis in some respects and are abundant in certain species and rare in others. Thus, they are very numerous in the rabbit's ovary, fairly frequent in the cat and absent in the domestic pig. Corner (1932) is of the opinion that there are none in the adult human ovary apart from the cells of the follicles and corpora lutea and explains the conflicting statements in the literature as due to the habit which some authors have of calling the theca cells of atretic follicles interstitial cells.

At this point a demonstration of sections of the ovary in ultraviolet light is helpful.

Popper's (1941) account will serve as a guide. Note in corpus luteum vitæ a fluorescent and non fading green or brown fluorescence in corpus atreticum a central tree-like fluorescence and in the stroma red brown and yellow colors. This line of investigation is likely to prove fruitful (Technique p. 76).

It is instructive to turn the clock back to Regnerus de Graaf, a young Dutch physician who in 1672 devoted himself, as others are doing today, to a systematic study of the female reproductive apparatus. The doctrine at that time was that females like males possess testes. They were called *testes muliebres* (L. *muliebris* relating to woman) and the tubes which carried the semen to the uterus were known as the *vasa deferentia mulierum*. In the uterus this female semen mixed with male semen and an embryo sometimes resulted. De Graaf concluded that the function of the testes muliebres was to produce ova and he referred to them as ovaries (L. *ovarium* an egg receptacle) and to the tubes as oviducts. He believed that the ova escaped from the follicles in the ovaries and passed through the oviducts to the uterus. Not until the studies of von Brer in 1827 was this finally proved. He wrote:

It remained for me to ascertain the condition of ova in the ovary for it seemed clearer than day that ova so small as those found in the tubes did not represent Graafian follicles expelled from the ovary, and I did not consider it probable that such solid bodies had been coagulated from the fluid of the vesicles. Now contemplating the ovaries before making an incision I clearly distinguished in almost every vesicle, a yellowish white point unattached to the walls which swam about freely in the fluid when the vesicle was pressed upon with a probe. Led on by a certain curiosity rather than moved by hope that with the naked eye I had seen ova in the ovaries through all the coats of the Graafian follicle I opened a vesicle and taking out a point in question on the blade of a knife I placed it under the microscope. I was overcome with amazement when I saw the ovule, now recognized outside of the tubes so clearly that a blind man could hardly doubt it. Surely it is strange and unexpected that an object so persistently sought for, and endlessly described as inextinct in every physiological compendium could so easily be placed before the eyes.

The above quotation is cited from Bremer (1936) who remarks that in this way the ova in mammalian ovaries which had long been believed to exist were first definitely seen within the follicles one hundred and fifty years after the discovery of the microscop spermatozoa the existence of which had never been suspected.

**Hormone Production**—Nomenclature of sex hormones has been considered (p. 324). Estrone also known as theelin is produced by the follicle cells hence another term for it is follicular hormone. It is the principal estrogen. Estrone or a substance indistinguishable from it can also be procured from plants (poplars, willows, pond lilies and cotton) as well as from lignite and petroleum (Dow, 1952). But the best source of estrone is the urine of stillbirths in which it is excreted in large amounts. For it to accumulate in the body would be the end of the process. In early life direction is given by chromosomal inheritance but we are all in a measure hermaphrodites. We have male and female attributes. The testes produce testosterone and estrone but the testosterone dominates more and more. The ovaries manufacture estrone and testosterone and the formation of estrone implies steroid metabolism. Unless there is an upset the individual is characteristically male or female for the remainder of his or her life. But working with certain amphibians Humphreys (1936) has shown that when ovaries are kept continuously from embryonic life onwards under the influence of testes they become reversed and change to testes. In humans however sex is more fixed. We are far removed from oysters who change their sex when convenient.

*Progestins* are manufactured by the corpus luteum which goes into action at ovulation but continues for some time its formation of estrone. Progesterone and secondary hormone and is only effective following estrone. When the developed ovum is not fertilized the duration of luteal function is limited but after fertilization some sort of signal is passed to the corpus luteum to continue its activity at least

inhibits further ovulation during the pregnancy, regulates the environment for the developing embryo and continues to excite the mammary glands. The structure of active and relatively inactive mammary glands is illustrated in figures 295 and 296.

The ovary is geared up by the pituitary (Fig 288). Follicle stimulating hormone accelerates production of estrone and luteinizing hormone that of progesterone.

**Testis and Ovary Compared.**—Though one is adapted to the production of sperms and the other to the manufacture of eggs there is an underlying similarity in the way the job is undertaken as well as in its hormonal regulation.

Both kinds of germ cells derive from the mesothelium covering the genital ridges in the embryonic body cavity. Both organs in the adult project into serous cavities, the scrotal and the peritoneal, but a lower temperature is required for the development of sperms than for eggs.



FIG 288—Ovarian changes in mice after implants of anterior lobe of pituitary. *A*, Normal, *B*, two anterior lobes from adult female rats implanted each day for four consecutive days, *C*, same treatment for nine days. (Smith, in Allen's Sex and Internal Secretions, Williams & Wilkins Company.)

This development takes place in special, rather isolated, tissue fluid environments. The degree of isolation increases as the later stages of spermatogenesis and oogenesis are reached. Thus, after the last division in the series, the spermatid matures as a sperm quite removed from the peritubular tissue fluid. Exchange of fluid for it to live must first be through the basement membrane and then through closely packed spermatogonia and spermatocytes, or perhaps more directly through vertically placed Sertoli cells. Still more impressive is the isolation of the egg within the Graafian follicle. Arriving and departing fluids must traverse the theca externa and interna as well as an array of granulosa cells. These intervening cells have been graphically referred to as "a bucket brigade." And the egg, within the follicle, has itself a thicker-looking cell membrane than the sperm.

The chromosomal changes in maturation are similar for both sperms and eggs.

insofar that the number of chromosomes is halved. But one half of the sperm carry an X chromosome and the other half a Y chromosome whereas all the eggs have an X chromosome. The sperms are of course shed in much greater number than the eggs because they are the ones that must find the eggs and billions fail to do so.

Androgen produced by the testis, conditions the male secondary sex organs and estrogens, formed by the ovary, enliven the female ones. Both organs are stimulated to make these hormones by pituitary gonadotropins of which there are two: the follicle stimulating hormone (F.S.H.) and the luteinizing hormone (L.H.) named from their action on the ovary.

Overproduction of androgen and of estrogens is curbed by similar acts of self-denial. Both tend to inhibit the production of the pituitary hormone which has the property of exciting greater production of themselves.

But the ovary has a function to stimulate for which the testis has no counterpart: namely, gestation. Therefore it produces a special hormone progesterone which among other things prepares the uterus for implantation of the fertilized egg. This hormone has a similar self-regulating action for (without influencing F.S.H.) it inhibits production of L.H. which as already indicated activates the corpus luteum cells to produce more progesterone.

Androgen maintains male secondary sex organs

Estrogen maintains female secondary sex organs

Androgen production stimulated by L.H.

Estrogen production stimulated by F.S.H.

Androgen inhibits production of F.S.H. and L.H.

Estrogen inhibits production of F.S.H.

increases production of L.H.

The fundamental similarity of these two organs is further exemplified by the fact that each can to some extent shoulder the duty of the other. Thus the testis can produce estrogen. The horse testis produces much estrogen. The urine of stallions contains about 8 units per liter of androgen and many thousands of estrogens. Some tumors of the testis are feminizing since they secrete estrogens. The ovary can manufacture androgen, for masculinizing ovarian tumors have been reported. Ovarian grafts when kept at a cool temperature produce androgens as shown by their maintenance of the seminal vesicles and prostates in castrated male mice in which they would otherwise atrophy for lack of androgen (Hill and Ganley 1936; Hill 1937). Moreover an ovary can be made to look more like a testis at least in rats in which Marx (1942) has induced partial transformation of Graafian follicles into seminiferous tubules by injection of testosterone.

Because there is considerable likeness in chemical composition between androgens and estrogens this ability of testis and ovary to produce both is not surprising. Androgen can be produced by the adrenal cortex and progesterone like substances in the adrenal cortex and placenta as well as by the corpus luteum. It is unnecessary to expand the list to show that some individual hormones like enzymes (p. 186) can be manufactured by cells in various organs which after all do not look much alike though they are basically similar in this common property.

**Fallopian Tubes** — These are the ducts of the ovaries (oviducts) and the oviducts in the body which are not attached to the glands they drain. This strange discontinuity between ovaries and Fallopian tubes is a fundamental feature in the architecture of the female reproductive system and harks back to conditions in

our remote ancestors. It is in the Fallopian tubes that fertilization normally takes place so that their structure and function is a matter of considerable importance

Each tube begins laterally near the ovary and runs in the upper edge of the broad ligament to the uterus (Fig. 281). The external shape is familiar to all students working in the dissecting room. The tube is divisible into a trumpet-shaped opening or *infundibulum* (*L. infundibulum*, a funnel) which is described as fimbriated. This leads medially into a dilated portion, the *ampulla*, which gradually narrows to the *isthmus* and expands slightly as it passes through the uterine wall

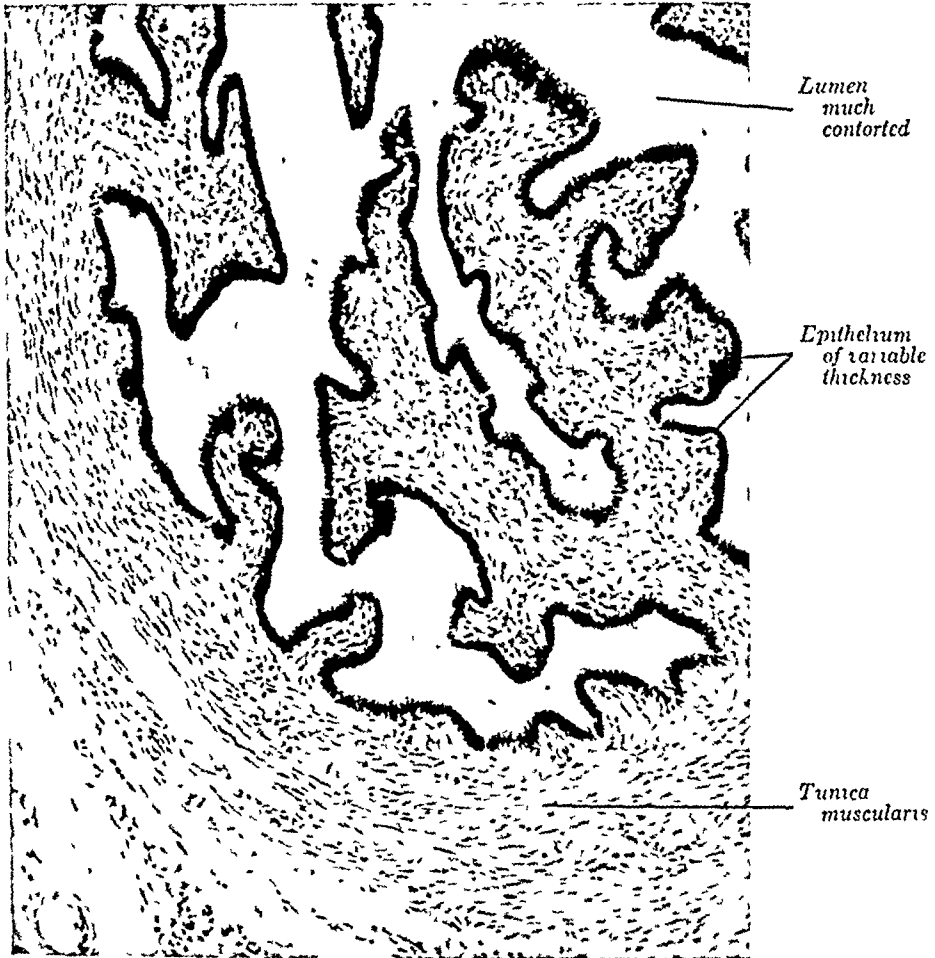


FIG 289.—Fallopian tube of thirteen year old white female with Banti's disease. Post-operative death from hemorrhage. Autopsy three hours postmortem. (Dept Pathology, Washington University No 10228, tissue obtained from Dr R E Stowell)

It will be observed that the mucous membrane is folded to form a series of gutters converging toward the uterus, but the length of each groove is probably shorter than is represented. Cross-sections of the ampulla look therefore quite different from those of the isthmus or uterine portion. The lumen is wider and occupied by an extremely plicated mucosa. The epithelium throughout is simple columnar or pseudostratified. Novak and Everett (1928) recognize three types of cells: secretory, "peg" and ciliated. As the term suggests, the peg cells are wedged between the others and are accepted as stages in their life cycle. The ciliated cells are shorter than the secretory ones. Lucas (1930) has confirmed and extended previous



work showing that the course of the ciliary stream is unbroken passing between and around the groups of non-ciliated cells. The lumen tube is not completely ciliated as Parker (1932b) states for the ciliated cells become relatively less numerous as the uterus is neared. The cells are tallest during the period of ovulation and shortest at the time of menstruation, which means that the secretory product as well as the ciliary motive power may vary. There is also a cyclical change in intracellular fat which is most abundant in mid interval (Butomo 1937). The epithelium is separated from the muscular coat by a little loose connective tissue which extends up into the folds (fig. 289). In the muscle circular fibers predominate but there are a few fibers that run longitudinally. These occur as isolated bands which are not all closely applied to the wall of the tube. Some are placed at a distance from it in the broad ligament which carries the tube in its free upper margin. Consequently, transverse sections often show that one side of the Fallopian tube is covered with peritoneum. Though the isthmus is about the same size as the ductus deferens and the ureter its structure is unmistakable. The epithelium of the ductus and ureter is not ciliated. It consists of two strata of cells in the ductus and of several in the ureter where it is limited on the luminal surface by large bloated 'transitional' cells. The ductus is most strongly muscled. The Fallopian tube alone lacks an inner longitudinal layer of muscle. The outer longitudinal muscle of the ureter and ductus does not spread into the surrounding tissue in the same way. Only the part of the ductus passing over the external iliac vessels is covered with peritoneum. The ureter like the Fallopian tube runs for a considerable distance just beneath the peritoneum.

The passage of eggs into and down the tube and the movement of sperm up the tube from the uterus to meet them has been investigated from almost every conceivable angle. See Hartman's (1932) critical review. The ciliated fimbriae stretch out like fingers toward the ovary. They are muscular and can move slowly just as the ovary can. The infundibular opening and the rupturing follicle may also be brought closer together by a vascular engorgement and swelling of the parts. Westman (see Hartman) fixed the ovary and fimbriae 2 to 3 cm. apart in experimental animals. 3 out of 6 of which nevertheless conceived and brought forth young. Evidently the distance is not so great that a margin of safety is wanting. The rate of movement of the egg along the tube doubtless depends upon the consistency of the secretion in the tube, the diameter of the tube, muscular contractions and ciliary action. The ciliary movement is toward the uterus. It is unsafe to make any pronouncement but in estimating its rôle the papers of Parker (1931) and Luers (1932b) should be consulted. The possibility must be borne in mind that the primary duty of the cilia may be one of clearance, as in the respiratory tract. The peristaltic waves in the tubes have been directly observed by roentgenological examination after filling with iodized oil (see Jarcho 1931). Normally they proceed toward the uterus and may well be the principal factor in propelling the eggs, but the direction may be reversed. The migration of ova through the tubes is delayed by excision of the corpora lutea (Corner 1928) and accelerated by injection of pituitary extract or pregnancy urine (Wislocki and Snyder 1933). Just how the sperm make their way upward is uncertain. The old concept that they remain a long time in the tubes has to be modified. The maximum is probably two or three days instead of two or three weeks, since the increase in temperature above that of the testis and epididymis soon kills them. That the time of survival may depend upon cyclic changes in uterine secretions is shown by Kugota (1929).

**Uterus.**—The uterus, a large pear-shaped sac, with well developed blood vessels and lymphatics (Fig. 290), in the wall of which two layers are recognized—endometrium and myometrium—is covered externally by connective tissue and peritoneum

The *endometrium* (G *endon*, within + *metra*, uterus) consists of epithelium and tunica propria. Its structure is very different in the successive phases of the menstrual cycle, which is to be regarded as a regularly recurring preparation of the wall of the uterus for implantation of a fertilized ovum. When this does not occur the state of preparedness cannot be maintained and the highly vascularized epithelial stratum, with the nutritive secretions elaborated by the uterine glands, is carried away accompanied by considerable bleeding. This is followed by repair and renewed preparation on the principle of better luck next time. The cycle is



FIG 290 —India ink injected lymphatics of muscularis of pregnant uterus of rhesus monkey seen from surface after removal of serosa and outer muscle fibers. On right is a typical collecting lymphatic passing toward broad ligament.  $\times 3\frac{1}{2}$ . (From Wislocki and Dempsey, courtesy of Anat. Rec.)

variable but generally lasts from twenty-five to thirty-two days. For convenience of description it is arbitrarily divided into periods. Unfortunately there is little agreement as to their terminology. The only sudden change in the whole process is the appearance of the flow from which the days are always counted. The following account is based primarily upon Bartelmez's monograph and review (1933 and 1937), following his earlier account (with C. M. Benslev) of the cytology of the mucous membrane (1932).

1. The *proliferative period* begins with the fifth or sixth day and lasts through the fourteenth day. It completes the postmenstrual repair. A section of the mucous membrane on the eleventh day is presented in figure 291, A. The surface epithelium is simple columnar in type. Three varieties of cells are present in order of frequency, secretory, ciliated and rod cells, but these cannot be distinguished at the low magnification of the photomicrograph. The extraordinarily large number

of mitoses is also not represented. Secretory cells are by far the most numerous. This period of rapid growth hyperplasia is controlled by the follicular hormone theelin. The red cells are perhaps comparable to the peg cells of the gall pan tube. The ciliated cells bear toward the vagina and their function is probably that of clearance. Uterine glands penetrate into the tunica propria from the surface and are lined by similar cells though the proportion of ciliated ones is somewhat reduced. A good account of the changes in the glands in the menstrual cycle has been given by O'Leary (1929) who has isolated individual glands and studied their morphology (Fig. 203). In the stage under discussion (Fig. 201) they grow so rapidly that they stretch into the tunica propria like fingers and elaborate secretion of a thin fluid containing little coagulable material and no glycogen. Their extremities are somewhat contorted and contribute to the formation of a stratum that stains deeply, on account of greater density of connective tissue and is called the *basalis* in contrast to the lighter and more superficial *functionalis* which undergoes significant changes in menstruation. It is the latter which is being regenerated for the *basalis* is not desquamated to the same extent. In early proliferative stages the functionalis contains only sprouting capillaries, a few mesofollicles follow later. Neutrophilic leucocytes and lymphocytes are present in the tunica propria in this and all stages of the cycle sometimes in great numbers. To what extent however they may be merely indicative of infection. There is much reticular connective tissue and practically no fat. The metabolism is so high that all fat is oxidized.

2. The *progravid* period is generally from the fifteenth to twenty-eighth day. This is illustrated in figure 201 B and C. The glands enlarge (B) and change into wavy ribbon like structures which now form secretion II rich in glycogen. This is followed by a marked increase in thickness of the mucosa (C) and a distention of the glandular lumina. These changes are under the control of estrogen (progesterone) and can be induced experimentally only after proliferation of the endometrium resulting from the action of theelin. A second zone, the stratum compactum can be distinguished just beneath the surface marked by freely staining tissue containing much fluid. The designation compactum seems to be a misnomer as far as the gravid stage is concerned. The blood vessels are contracted and the capillaries have given way to spiral arterioles and venules. The former continue to grow in the gravid stage for Bartelmez has found many in their walls in a twenty third day specimen. The tissue illustrated in figure 201 C was removed the day before an expected period. Fertilization for implantation of a fertilized ovum was complete. If one is received at this stage the mother gives of its nest and enlarges into a decidua at the same time inferring the egg and in the ovary by some unknown chemical messenger travelling to the uterus to continue its activity and produce corpora and finally in some animals relax. If not menstruation follows.

3. The *Menstrual Period* (First to Third or Fourth Day). Figure 202 (a, b, c) are from tissue removed on the second and third days respectively. It will be observed that the functionalis is crumbling away while the basalis is being altered. Why this disintegration should occur is not very clear. Perhaps it may be traced to some peculiarity or weakness of the blood vessels. There is usually on the first day a marked accumulation of leucocytes. Afterward a mass of lymphocytes follows and both cell types degenerate in large numbers. If the basal cells, leucocytes and blood are discharged, but the basement membrane

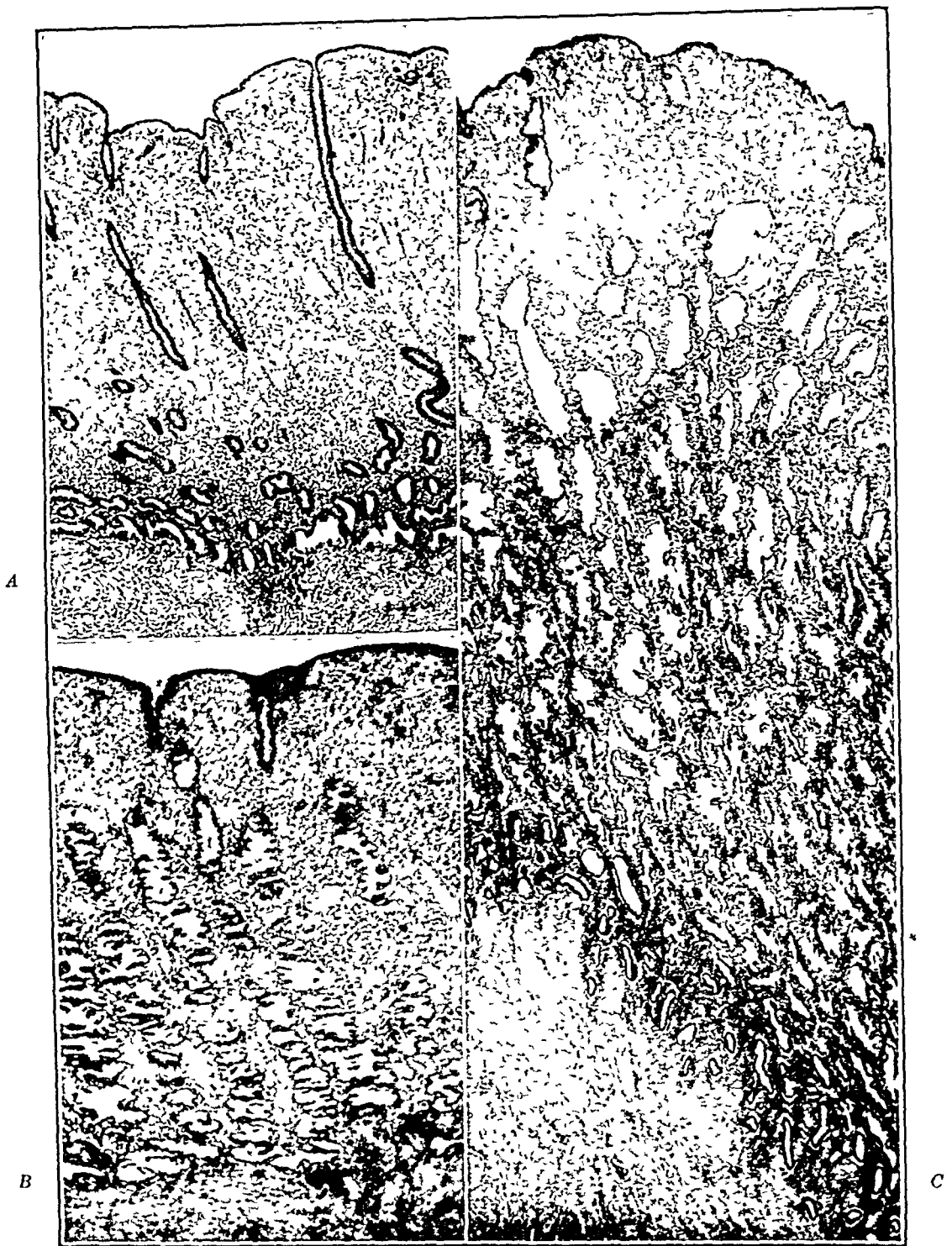


FIG. 291 —Stages in menstrual cycle continued in figure 292. *A*, *Proliferative*, eleventh day. Mucous membrane, 2.1 to 3 mm thick, largely reorganized so that only basal zone stains deeply. Surface epithelium mostly columnar with frequent ciliated cells. Arterioles and venules differentiating in superficial third where reticulum is almost completely developed. Glands straight except for lower ends. Type I secretion but traces of glycogen. Mitoses very abundant (O'Leary, *Am J Anat*). *B*, *Progesterone*, twenty-third day. Associated with a corpus luteum. Mucous membrane, 2.8 to 4.8 mm. Glands meandering and sacculated down to muscle. Type II secretion with abundant glycogen. Tunica propria edematous. Arterioles reach to surface epithelium. Mitosis (Bartelmez, *Contrib to Embryol*). *C*, *Progesterone*, twenty-eighth day. Associated with a corpus luteum. Just before expected period. Mucous membrane, 3.7 to 4.9 mm. Glands distended with Type II secretion. Stratum compactum indicated by edematous outer zone (Bartelmez, *Contrib. to Embryol.*)

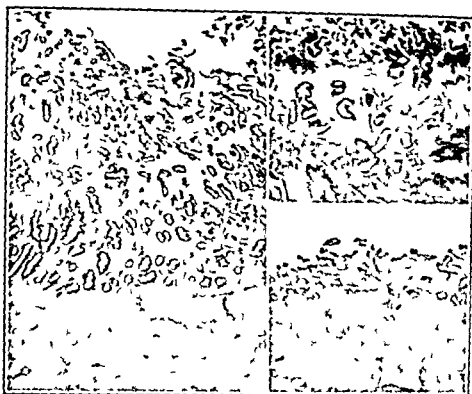


FIG. 292. Stages in menstrual cycle continued from Figure 291. A. Menstruation second day. Surface epithelium still intact in places. Outer coiled ruptured coiled of progesterone type. B. Menstruation third day. Mucous in mid canal of 1-1 mm. Surface detached. Gland irregular in form with dilated extremities. Strong dilation of 1-1 of menstruation. In some regions glial epithelium is present, giving the surface to repair it. Mucous in mid canal 0.5 to 1.0 mm. Glands irregular show mucous secretory activity. (Bartelmez, Control; Lantieri.)

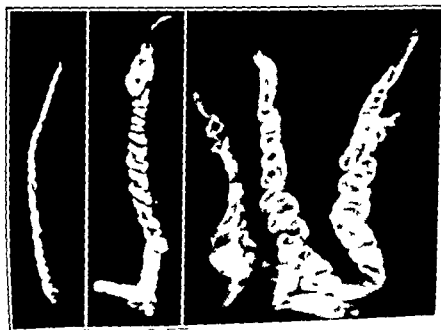


FIG. 293. Stages in menstrual cycle continued from Figure 292. A. Menstruation fourth day. B. Menstruation fifth day. C. Menstruation sixth day. (Bartelmez, Control; Lantieri.)

and checked by a peculiar reduction in the flow of blood. An ischemia (G. *ischo*, I keep back + *haima*, blood) sets in despite the fact that some of the arterioles may have open ends. This may be partly explained as a consequence of the peculiar structure and action of the arterioles. It has been found that they possess bands of longitudinal muscle fibers immediately beneath the tunica intima, generally on one side. The only other place in the body where this obtains is in the arteries

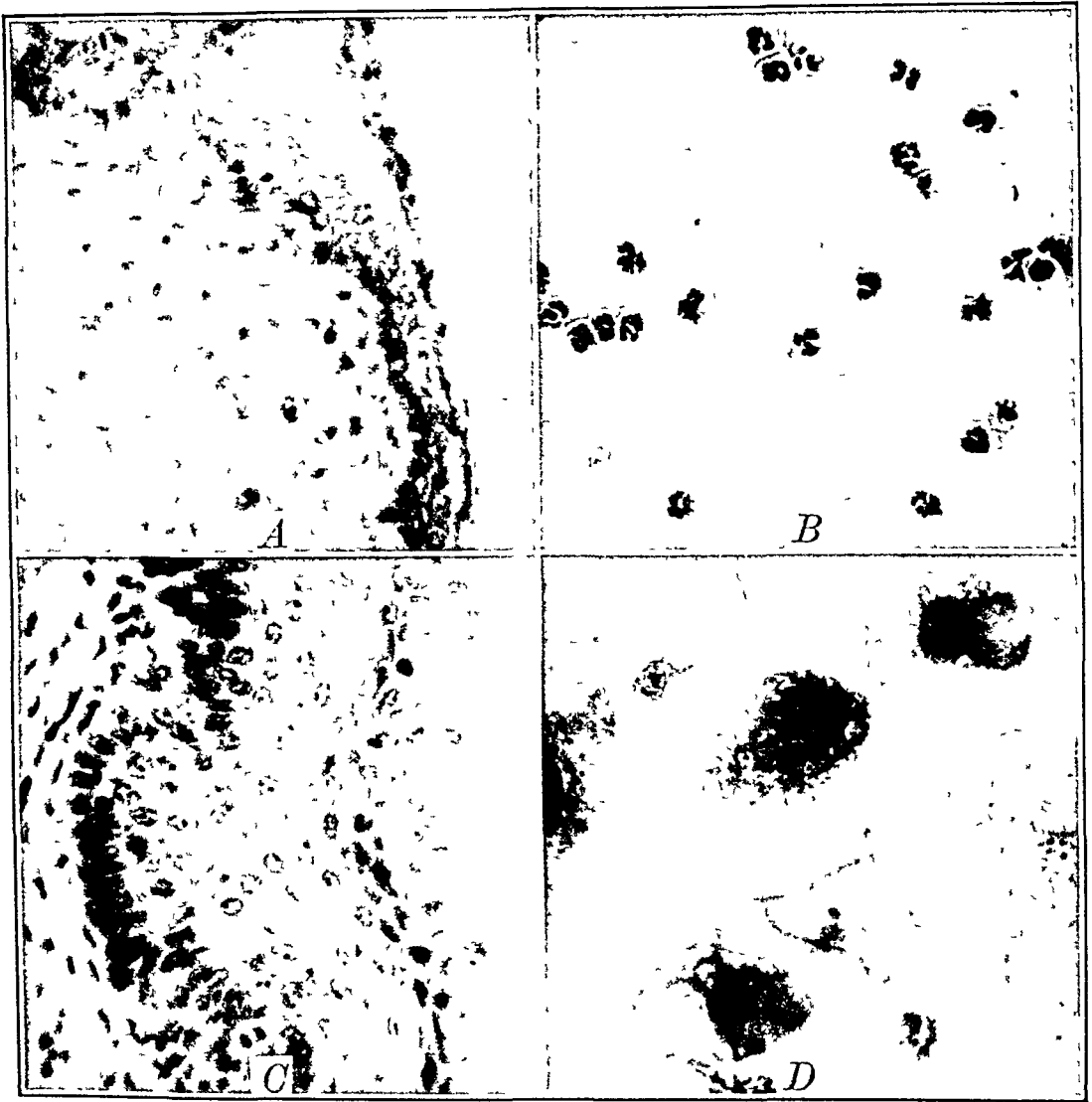
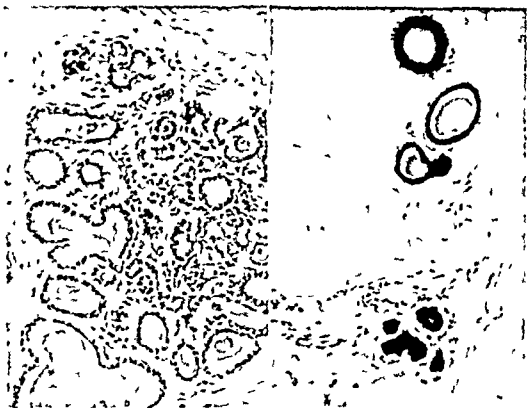


FIG 294 —Sections and smears of rat's vagina to show growth reaction from theelin. A and B, Control, ten days after double ovariectomy. C and D, Ovariectomized animal, ten days after first series of theelin injections. (Allen, Sex and Internal Secretions, Williams & Wilkins Company.)

of the erectile tissue of the penis. Maintenance of normal tone of this muscle may, as in the penis, produce bending of the vessel and reduction of blood flow, while relaxation facilitates blood flow. It is possible that with degeneration and the liberation of toxic substances nerve terminals are stimulated, and that on reflex contraction of the longitudinal muscle contortion of the vessel wall and decrease in width of lumen result. However, this may be, it is certain that the arterial

system is in some way blocked because fluid injected through the arteries does not escape into the surface, which happens easily after venous injection.

4. *The Lactation and Period (Fourth or Fifth Day).*—The fourth day is represented in figure 262 C. The densification of the surface is even greater but repair is beginning, though it cannot be seen in the photomicrograph. It has been demonstrated that the epithelium of the basal parts of the gland, which were not lost, spreads out over the surface, soon covering it completely. New blood vessels form, the mucosa thickens and the proliferative stage which we started with is reached.



A

B

FIG. 262. A. Lactating breast of thirty-five year old white female. (Photomicrograph taken by Dr. P. F. Stowell, Dept. Pathology, Washington University, No. 10116.) B. Lactating breast of twenty-five year old white female with periparturient mastitis. At extreme end, quarter of an inch from stem. (Dept. Pathology, Washington University, No. 10116, lect by Dr. P. F. Stowell.)

tissue, nourished by arteries and innervated by nerves. The functional anatomy of the uterus is presented *in extenso* by Ivy (1942).

**Vagina.**—The vagina is a distensible tube, lined with stratified squamous epithelium, a lamina propria containing much elastic tissue and coated with muscle, which leads from the uterus to the exterior. At first sight an isolated section may look something like the skin. Though the epithelial cells contain keratohyalin, the surface is moist and not ordinarily cornified. Cornification, however, results from lack of sufficient vitamin A in the diet. The tunica propria often contains an abundance of lymphocytes, perhaps even lymph nodules which are never found in the dermis through which absorption is very much less. There is no counterpart for the sudoriferous and sebaceous glands of the dermis. The only glands present

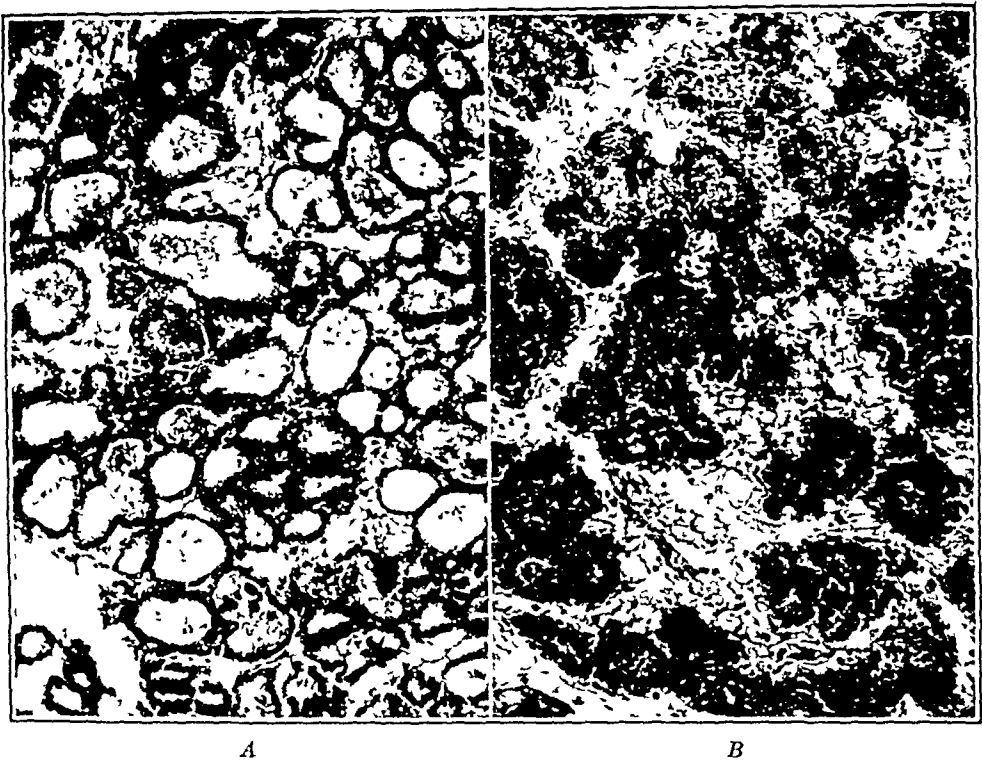


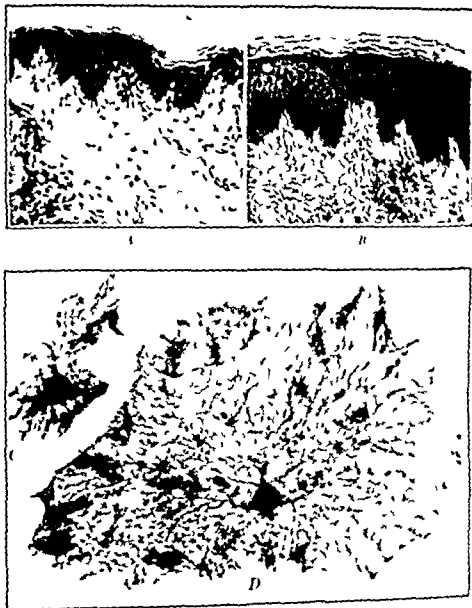
FIG 296 —Physiological changes in mammary glands of white mice. *A*, Stimulated to activity after parturition, *B*, involuted five days (Turner and Gomez, Univ. Missouri Agric Exper Station, Research Bull.)

are a few of the mucous variety in the vaginal *fornix*, a kind of blind pocket, behind the projecting uterine cervix. They are of the cervical type. Neither are there any sense organs like those of Meissner. The interlacing of smooth muscle fibers, the longitudinal ones of which are most numerous, is typical. Striated fibers form a kind of sphincter at the opening. The fatty areolar tissue which underlies the epidermis is absent.

Like the other accessory reproductive organs, the vagina is held in good condition by adequate supplies of estrone (see Fig 294) and vitamins. It exudes a viscid fluid which contains mucus derived from the glands of the vaginal fornix and cervix and secretions and cells from the uterus. The systematic study of vaginal smears of the guinea-pigs by Stockard and Papanicolaou (1917) afforded data by which rhythmic alterations in the ovary and uterus could be accurately timed and



related. Without delay attempts were made to utilize histin vaginal smears for the same purpose. Leucocytes, lymphocytes and epithelial cells of different sorts were diligently counted, but cyclic changes were less concise than in the guinea pig, rat and other animals investigated. A well illustrated description of histin smears is that of Papanicolaou (1925).



in the sexes is that the urinary tract is not utilized in the female for the discharge of sexual products. The clitoris corresponds embryologically to a part of the penis. It is made up of two erectile bodies and a rudimentary glands. The vestibule is equipped with small glands not unlike those of Littré and with two larger ones, the glands of Bartholin, which secrete a lubricating mucus. The latter resemble the male bulbourethral glands.

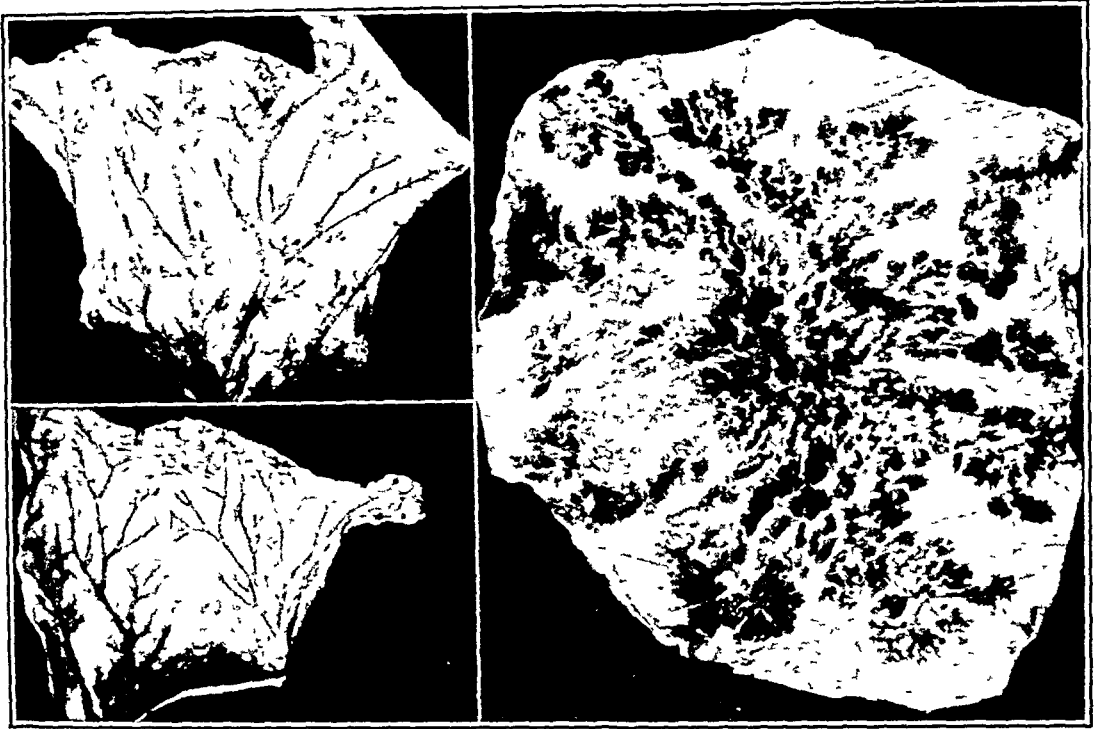


FIG 298 —Showing effect of theelin and corporin on the mammary glands of rabbits. A, After preliminary action of theelin causing growth of duct system of a castrated rabbit, corporin was injected for twenty days. The ducts showed shrinkage in diameter and there was no lobule proliferation. B, A second check gland removed thirty days after the corporin extract injection. Note further involution of ducts. C, Mammary gland of a castrated rabbit after 18 daily injections of theelin and corporin together resulting in an extraordinarily luxuriant growth of lobules  $\times 2\frac{1}{2}$ . (Turner in Allen's Sex and Internal Secretions, Williams & Wilkins Company.)

**Mammary Glands** —These are of ectodermal origin. They are developed as ingrowths of the surface epithelium. In the adult they constitute large, branched, tubulo-alveolar structures which discharge by several ducts at the nipples. The appearance of the secretory tubules and alveoli is seldom uniform throughout the gland and varies profoundly in different stages of functional activity. When the lumina are dilated with secretion (Fig. 295) a section may look something like the thyroid for the continuity of the spaces is not evident. The distinctive features of the mammary glands are abundance of fat in the lumina and within the secreting cells and strands of smooth muscle in the interstitial tissue. Moreover, the distal margin formed by the cells is irregular and ragged. Some of them project much farther into the lumen than the rest. Inactive glands (Figs. 295, 297), on the contrary, are very different from the thyroid. The lumina are collapsed and the interstitial tissue is increased in amount. In humans this increase is largely made up of fat. The cytology of the process of secretion has been investigated by Weather-

ford (1929) Jeffers (1933) and many others. Fatty and albuminous materials are heaped up in the distal cytoplasm and make their way into the lumen by the usual obscure way. The old idea that the secretory act consists of extrusion of cells is not valid although some cells are cast away and leucocytes and lymphocytes enter the lumen sometimes in large amounts especially when the drainage of secretion is blocked.

The activity of the mammary glands is cyclic and hormone-regulated. It exhibits distinct individual differences. Subjective symptoms of a first change in the mammary glands during the menstrual cycle are often marked. Loeb (1932) thinks that the histological evidence of a slight but noticeable proliferation of new secretory acini in the progestin phase is satisfactory. Allen (1937) has observed an enormous hypertrophy of the skin of the nipple and of the glands itself in ovariectomized monkeys after injections of theelin. The extent of the alteration is indicated in figure 297. Furner (1932) has found that the mammary gland of the rabbit responds similarly but to a less degree to theelin and corticosterone development under the action of corporin. The former causes a growth of ducts and the latter of secretory lobules. Both are necessary. When a series of corporin injections was given after the theelin had produced an increase in the ducts lobule proliferation was not obtained (fig. 295, 1). Thirty days later there was a noticeable atrophy (2). When theelin and corporin were injected together there was a conspicuous increase in ducts and secretory lobules (3). It is unsafe to assume that the mammary glands of different species to be regulated in the same way. Active secretion occurs normally at the conclusion of pregnancy and may be caused experimentally by injections of extracts of the pars distalis of the pituitary containing the so-called lactogenic hormone (p. 141). Since this is effective in ovariectomized animals it may be concluded that the principle acts directly on the mammary gland. They are also activated in pregnant rats by hyperthyroidism (Weichert and Boyd 1931). When lactation is beginning and when it is tapering the milk produced is of a different quality. It is poor in fat but contains more protein and many large granular colostrum bodies as well as desquamating cells and leucocytes.

Benign and malignant tumors are almost as common in the female mammary gland as in the prostate. This is not to be wondered at. The glands are subjected to regular monthly waves of activity which do not lead to expulsion of milk and are followed by regression and to prolonged periods of heavy development with each pregnancy ending likewise by regression or involution as it is more commonly called. But the return to the previous relatively passive state is probably incomplete. Each spurt of activity whether short or long leaves its mark on the living cells that remain. The glands are tortuously dilated here and there and often constricted so that drainage is sluggish at the end of lactation and wholly lacking in the menstrual periods. Retention and concentration of product with the development of many different sorts of local environment is typical of the mammary glands as it is of the prostate. The waste is cast off as in the menstruating uterus and a similar opportunity is not given to set up a new mechanism. That many irritating substances must be present in the diffusion through the tissue is evidenced by the lymphatic effusions of the interstitial tissue so often met with. Evidently the epithelial cells are not so well adapted to the influences in addition to trauma for the breast are not so well

structures Nature, however, guards against mechanical injury by the peculiar sensitivity of the nerve supply

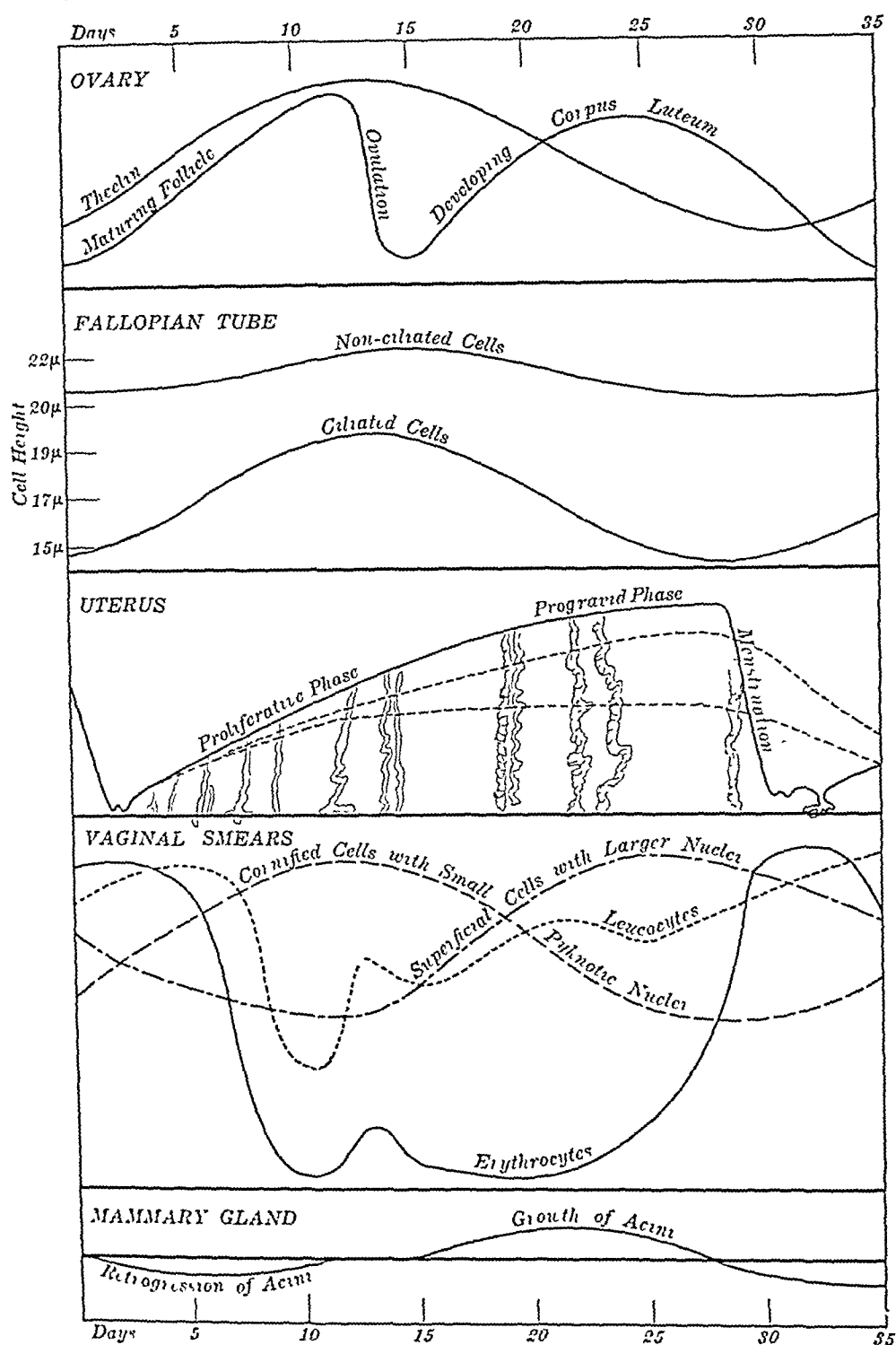


Fig 299 —Chart indicating possible correspondence in time between cyclical changes in the female reproductive system (The graphs for the Fallopian tube are from Lucas, after Tietze, and those for the vaginal smears were supplied by G. N. Papanicolaou.)

**Integration of Activities.** — Figure 299 is intended to indicate what little is known of the correspondence in time between cyclical changes in the female reproductive

apparatus in the absence of pregnancy. It is unsafe to assume that because this occurs that happens in animals the same occurs in humans. Even the human cycle is subject to considerable individual variation.

In the *ovary* a maturing follicle usually ruptures or ovulates sometime between the twelfth and fifteen day after the menstrual flow appears externally. The ovum is washed into the Fallopian tube and the collapsed follicle develops into a corpus luteum. This enlarges but unless the ovum is fertilized and becomes implanted in the prepared uterine mucosa of the progravid stage it regresses to form a scar-like corpus albicans. As indicated by the curve the manufacture of the follicular hormone, theelin, does not cease abruptly with the rupture of the follicle but is continued to some extent by the corpus luteum.

The ciliated and non-ciliated cells lining the *Fallopian tube* increase in height up to the time of ovulation when the ovum is received and then decrease progressively until the flow begins. Since most of the latter are secretory this is to be interpreted as a well timed storage and discharge of the necessary fluids.

Following the preceding menstruation the mucous membrane of the *uterus* passes through orderly phases of repair, proliferation and progravid preparation for the receipt of a fertilized ovum. The glands are at first straight and almost circular in cross-section. Their diameter increases and they become contorted elaborating a secretion which in the progravid stage alters and becomes very rich in glycogen. Unless however implantation of an ovum does take place and the mucosa in some unknown way so informs the ovary, menstruation results and the outer part of the epithelial bed engorged with blood and nutritive secretions is swept away. The dotted curves in the graph are intended to indicate that the increase in thickness of the mucous membrane may not be as great as represented and that menstruation may be less sudden, less destructive and of longer duration.

Alterations in the cells observed in *vaginal smears* are equally cyclic with changes in the ovaries, Fallopian tubes and uterus and can be much more easily studied. The curves presented give information as to sequences and to changes coincident with ovulation but not of the relative percentage of cell types. That leucocytes and reds are discharged in large numbers during menstruation is evident but Papanicolaou is alone in reporting some reds each day through the interval.

Rhythmic changes in the *mammary glands* are often subjectively noticeable. There is reason to believe that a slight growth of secretory acini takes place as the uterus becomes progravid probably under the influence of theelin secreted by the ovarian follicles or of the luteogenic hormone of the pituitary. If pregnancy does not supervene this is followed by a low retrogression. The immediate driving force appears to be identified in some way with the maturation of ovarian follicles and the discharge of ova though it must be admitted that menstruation can occur without ovulation and the gonadotropic hormone of the pituitary has to be reckoned with.

**Nervous versus Hormone Regulation.**—Almost every phase of the female reproductive cycle is hormone regulated. The smooth muscle so widely distributed in the system is by nature involuntary. Conception can take place and pregnancy be completed after section of the spinal cord. Menstruation is not prevented by cutting the nerves to the uterus and ovaries. It occurs even in sections of monkeys' encephalotomies transplanted into the anterior chamber of the eye (Markoe, 1940). Normal reproductive functions can apparently be carried out by animals after complete sympathectomy. Yet evidence keeps coming out for the

presence of a sex center in the hypothalamus. According to Hartman (1939) it is certain that sexual stimuli from various parts of the body flow through the hypothalamus to the pituitary where they increase the output of gonadotropic hormones.

**Placenta.**—Space does not permit reference to structural changes during pregnancy but the placenta which unites fetus to uterus should be mentioned in passing. Placental villi from the fetus burrow into the uterine decidua. The invading cells are of somewhat irregular size and are said to look more malignant than any others normally present in the body. The association between fetal blood in the villi and maternal blood surrounding the villi is very close (Fig. 300). It is not surprising that the baby shares for some months its mother's immunity to certain diseases. Radiosodium has been employed by Flexner and Gellhorn (1942) to measure rates of placental transfer. Estrogen, gonadotropic hormone and progesterone have been reported as being produced by the placenta. The case for the last named is the most satisfactory.

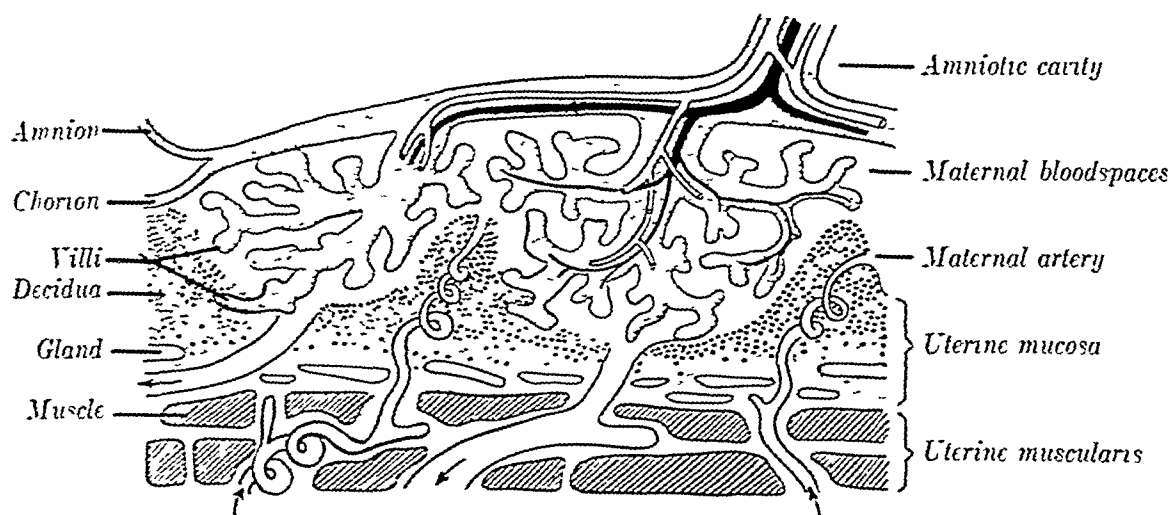


FIG 300 —Diagram illustrating close association of maternal and fetal blood in the placenta. (Redrawn from Bremer, Textbook of Histology, courtesy of P. Blakiston Son & Co)

## SUMMARY

Reproduction is one of the most necessary of all vital activities, for individual living units cannot last for ever. The stream of life must be maintained whatever the environment and habits of life of the organism. Consequently this system, and particularly the female one, presents a wide variety of special adaptations. In humans the eggs possess a good deal of energy stored in the cytoplasm which, however, is very much less than that with which forms below the mammals are endowed. Thousands of eggs are produced only to die in the ovaries, while hundreds are made available for the sperms in the Fallopian tubes. But the eggs do not lie in wait for any considerable time. Hartman (1932) remarks, "What a fertile organism the human species would be if this were the case. The marvel is not how fertile but how sterile is humanity." Rhythm is likewise a fundamental vital attribute and it is better exemplified by this repeated preparation for pregnancy than by any other process. To provide it, Nature has surpassed all her other efforts in endocrine regulation. This has been described from the standpoint of progressive evolution by Allen (1932). To the primary sex hormone, estrone, she has added others with definite duties. The nicety of the adjustments and the interlocking of

the processes captures the imagination but it has been repeatedly observed that the hormonal integration is designed to meet the peculiar and special requirements of different animals. It would be inviting trouble to suppose that any of the female sex hormones act with the constancy and uniformity in different orders and classes that thyroxin does. The female reproductive system has many requirements. Vitamins E and A are important and one gains the impression that the role of the nervous system has been rather neglected by reason of the very natural concentration of attention on the endocrines. Yet the primary and all the accessory organs are richly equipped with smooth muscle. It is a dynamic not a static system.

Like the male reproductive system the female one provides the genes which lead to the development of hereditary traits but it does more: it fashions the offspring by extrachromosomal factors. The cytoplasm of the ovum is very influential. Thanks to numerous pioneering studies under the direction of Dr. C. C. Little in the Roscoe B. Jackson Memorial Laboratory at Bar Harbor summarized by him (1944) it is clear that the uterine environment plays a part much greater than was formerly suspected. This extends even to the skeletal system. When fertilized ova of mice of a strain having five sacral vertebrae are transferred to the uterus of a six sacral vertebra strain the number of sacral vertebrae in the developing embryos is increased (Little 1943). And mother's milk makes a difference. Mice of a high immunity cancer strain have a low incidence of this disease if given only milk of a low cancer strain and vice versa (Buttner 1942). A milk influence in humans has not been proved but it should be sought. It is fundamental to remember that at these three levels—egg cytoplasm, uterine environment and nursing, extrachromosomal factors are to be expected.

The female reproductive system unlike the male one miraculously ages relatively quickly. The changes have been graphically described by Edgar Allen (1947). Nature the master builder has run great risks to accomplish her ends (p. 10). In using estrogens materials of great service are employed but they are perilously close to chemical carcinogens and can produce cancer in experimental animals.

## CHAPTER XX

### SKIN

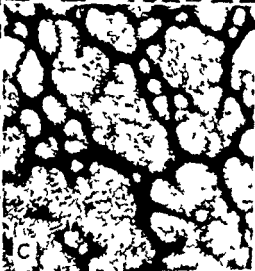
HAVING described the systems of the body, with due emphasis on the blood vascular as the principal integrator, it is natural to conclude with the skin which encases all the living tissues and organs, unifying them, protecting them from the environment and informing them by its sense organs of changes in the outside world to which they must adjust themselves if the individual is to survive. In the space of a decade or two the science of dermatology has undergone a renaissance comparable to the discarding of old notions and the discovery of new facts about the female reproductive system, but for different reasons. Allergy has become fashionable, as well as important, and in it the skin plays the star rôle. Many of us, who have become virus conscious, find that the skin is affected by many of these intriguing disease-producing agents. The appearance of the skin has become recognized as a by no means to be ignored indicator of the condition of the body beneath. Millions of dollars are spent every year in disguising it. Dermatology is included by Gregg (1940), Director of the Division of Medical Sciences of the Rockefeller Foundation, in his considered estimate of fields of medicine ripe for development.

Orientation should be given to microscopic study by making a preliminary survey of living skin all over the body. Begin by comparing movable skin with skin firmly anchored to the underlying tissue and skin in exposed and protected situations. With the help of a hand lens examine the surface pattern, hairs, scars, moles, etc. Numerous illustrations provided by Wolf (1940) will be of assistance. Obtain an idea of the thickness of the avascular epidermis by sterilizing the back of the hand with alcohol and cutting small thin slices parallel to the surface with a razor blade until a trace of blood appears. Correlate depth with sensitivity, bleeding and microscopic structure. Pull out hairs. Examine them microscopically and the hair pits with the hand lens. The pits are likely sites of invasion by microorganisms. Very revealing is examination in ultraviolet light. "Blackheads" show in different individuals remarkable red, white and yellow fluorescence as described by Figge (1942). Some infected hairs fluoresce green. Scars, pigment, lipstick, true and false hair and teeth, the tongue and other structures fluoresce in characteristic ways.

**Epidermis.**—This sheet of tissue, enshrouding the body, is thinner than we usually realize. Except in a few areas, like the palms of the hands, it is so thin that if seen directly from the side it would be quite invisible. The thickness of the epidermis in the antecubital fossa of young males measured in vertical sections is about  $34\ \mu$  (Evans, Cowdry, Nielson, 1943). Making a generous allowance for shrinkage it is apparent that *in vivo* the thickness often is less than  $100\ \mu$ , which is about the limit of naked eye visibility provided that the presence of the structure is not betrayed by reflection or by differences in character of surrounding materials.

By soaking whole skin in dilute acetic acid the grip of the dermis is loosened so that the epidermis can be stripped off, stained and examined as a whole mount (Technique, p 69). Even a preliminary study of preparations of this sort brings to light altogether unsuspected *epidermal patterns*. Some of these, seen from within, are illustrated in figure 301. The tiny dark and gray spots are the hematoxylin stained nuclei of the epidermal cells. In "A" very thin epidermis is shown viewed from the inside. The duct of a sweat gland is included. In "B" thicker epidermis





projects in rounded papillæ up toward the observer, that is into the dermis. The epidermal sheet is continuous, but it is not everywhere photographed because the focussing was on the papillæ. In "C" a network of epidermal ridges is represented in the substance of which are circular, lightly stained areas of very thin epidermis. In "D" the epidermis is much thicker and there are areas where none is seen since they are out of focus. Curious rounded clumps of cells extend into them from the sides. In "D" still another pattern is illustrated of widely spaced ridges with fewer contained light areas.

The value of the study of whole mounts of epidermis is not simply the establishment of regional patterns, though these are expressions of special services performed, but in the discovery of early lesions which might well be overlooked if sections alone were examined. Since the nuclei in mitosis can so easily be counted in relation to the non-dividing ones, it has been possible for Cooper and Schiff (1938) to find that a *mitotic rhythm* exists with maximum frequency at night and minimum by day. This is probably the answer to the query so often heard. Why, if the epidermal cells are multiplying to make good losses from desquamation, do we find so few mitoses in sections of the skin? The ordinary practice of fixing tissues during the day obviously involves examination when mitotic frequency is at a low ebb.

Introduction of another method of loosening the hold of dermis on epidermis by heat (Baumberger, Suntzeff and Cowdry, 1942) permits the collection of epidermis in a condition suitable for chemical analysis. Thus is overcome a stumbling block to accurate determinations because heretofore variable amounts of dermis were included in the material analyzed. Already significant data on the chemical changes occurring in precancerous epidermis have been discovered (Carruthers and Suntzeff, 1943).

But *epidermal stratification* and some other phenomena can best be examined in sections. Students are often perplexed because they may not see in a particular section of the skin all of the strata described in textbooks. This is only possible when the epidermis normally is very thick, as in the palmar and plantar surfaces (Figs 302, 303).

1 *Stratum basalis*. This consists of epidermal cells actually in contact proximally with the basement membrane and consequently nearest to the dermal tissue fluid.

2 *Stratum spinosum*. Here there may be several layers of cells which are larger than the basal ones and are equipped with spine-like processes. The spines appear to join neighboring cells together, as is indeed indicated by microdissection (Thanhofer, 1933). These two strata are sometimes grouped together under the heading of *stratum germinativum* for cellular multiplication can take place in both of them.

3 *Stratum granulosum*. As the name implies this is a stratum in which the cytoplasm of the cells is loaded with granules.

4. *Stratum lucidum*. Here the granules seem to flow together with the production of a clear, lucid appearance.

5. *Stratum corneum*. In this layer, which may be wider than all the rest of the epidermis put together (Fig 301), the cells die, become dehydrated and keratinized.

With decrease in thickness of epidermis, in other regions, these strata fade out in the following order: first the stratum lucidum, then the stratum granulosum and finally the stratum spinosum. But only in very thin epidermises, as in those of mice, does one commonly find only a basalis and a corneum represented each by a single row of cells. As any very thin epidermis becomes thicker in response to new

demands or influences strata not previously present make their appearance. The stratum lucidum is the last to appear if indeed it does show itself for it has an evanescent quality.

Clearly we have to do with a *depression gradient* extending from the basement membrane to the free surface of the epidermis. The cells next the membrane are

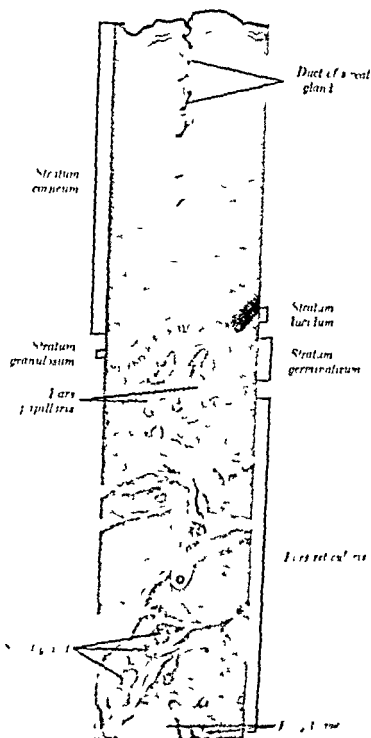


Fig. 5. Section through a cross-section of the epidermis of the skin.

most favorably placed for the receipt of necessary materials and for the disposal of waste. Those more removed are handicapped, while for others still farther away life becomes impossible. They die, but continue to serve as an essential dead, protective coating all over the body only to be gradually discarded as other cells rise and take their places. This gradient from the quick to the dead, from water rich to dried up cells containing much rather insoluble horny material, is only feasible in so thin a layer because the epidermis is everywhere avascular and the cells are so closely fitted together that fluid exchange is strictly limited. The plan of construction reduces epidermal tissue fluid to a minimum.

The factors involved in the steady maintenance of this gradient, within the different thicknesses of epidermis of the skin in various regions, are unknown. Also to be discovered are the factors which operate when a given epidermis thickens, comes to be made up of more cells, in other words, becomes hyperplastic. Somewhere in the epidermis *replacement* must be provided by a strain of vegetative intermitotic cells comparable to the so readily identified spermatogonia in the testicle. Where the epidermis is two cells thick it is certainly the basal cells immediately in contact with the basement membrane that on division produce some daughter cells which, like them, are vegetative and undifferentiated, while others, unlike them, are pushed a little nearer the surface and become differentiated. Without this reserve of vegetative cells the epidermis would soon differentiate itself out of existence owing to failure of replacement.

In thick epidermis cell division is not restricted to basal cells but includes many spinous ones. The zone of replacement is wider. To localize in this zone the layer in which cell multiplication is at a maximum is difficult. Some claim that it is in the spinous layer (Thuringer, 1928) not in the basal layer. Where this is in fact the case it does not mean that these spinous cells are here the vegetative intermitotics and that some of their descendants shift toward the surface to take the place of worn out corneal cells and that others move inward to replace basal cells. On the contrary, it would simply indicate that these particular spinous cells are differentiating intermitotics on a par with rapidly multiplying spermatocytes in the testicle and are derived from the basal cells as the spermatocytes are from the spermatogonia. In both situations it is the single layer of cells nearest to the underlying tissue fluid which carries on the line. Evidently the possibility must be entertained that the position of the level of maximum frequency of cell division may shift in both distal and proximal directions in different physiological and pathological conditions. This is more than an academic question because it is involved in our interpretation of benign and malignant epidermal hyperplasias.

That epidermis regenerates promptly, when some of the cells remain alive, is common knowledge. It is also well known that when all the epidermal cells are killed right down to the dermis the living cells from the side will spread quickly over the defect and form a new epidermis if the distance is not too great. No one, however, has managed to determine the time required for normal replacement in the absence of injury. The trouble is that there are no data on time consumed in an epidermal mitotic division, on life span of the differentiating intermitotics, on the number of these cells in the series, or on the life and death span of the final postmitotic corneal cells. The speed of regeneration after wounds and the length of life of epidermal cells in tissue cultures are not infallible indicators of time relations in normal maintenance.

In the course of development sheets of epidermis come together along "fusion

lines. Small masses, especially in the face, may be displaced into the dermis where they can maintain themselves submissa as fungoid nests for forty-five years or more until a few of them break out as tumors. The tempo of life for these serpentine cells must be unusually. In traumatic implantation, where epidermis is forced in-

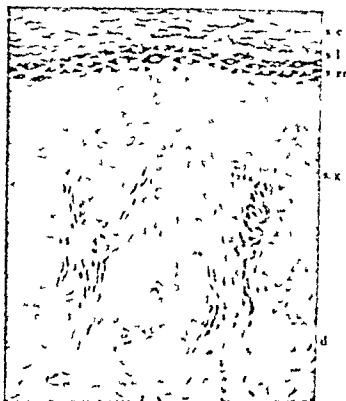


FIG. 337. Skin on the palmar surface of the finger. (Cowdry, after Sclafner Special Cytology, Paul B. Hoeber, Inc.)

dermis by penetrating wounds or even by the sweep of a razor blade, the displaced epidermis maintains itself but makes its presence known more promptly. Length of cell life is not unusually extended.



FIG. 338. Epidermis of black person in which chromatin is more compact than in white where it is less so; between the nucleus and the surface. (Cowdry, after Sclafner Special Cytology, Paul B. Hoeber, Inc.)

Some of the mineral constituents of both epidermis and dermis can be re-estimated as they undergo regeneration. The mineral skeleton within all of the cells

material burnt away, is illustrated in figure 316. This technique combined with histospectrography, is capable of demonstrating important facts. For instance MacCardle, Engman and Engman (1941, 1943) have found a cutaneous magnesium

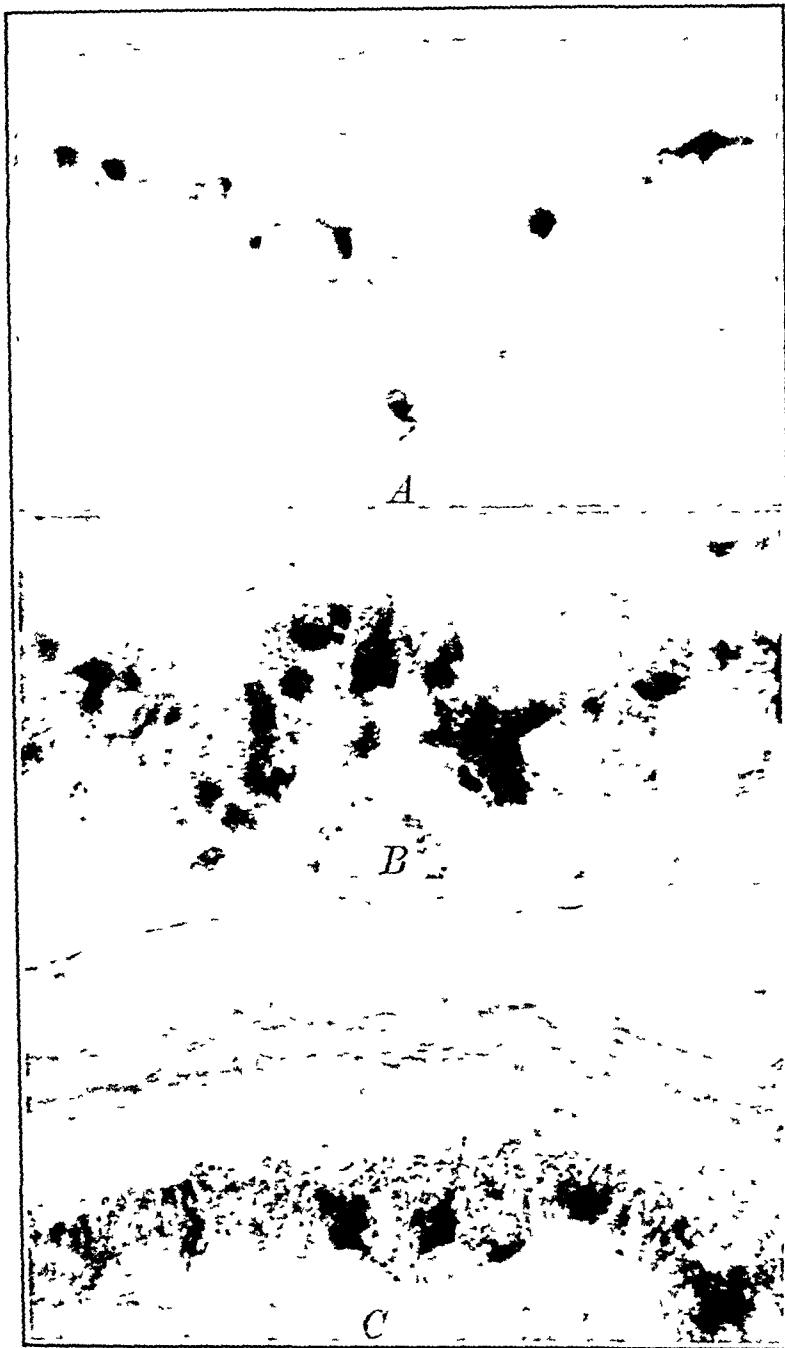


FIG. 305.—Dopa-positive cells in epidermis. *A*, From skin-normal Caucasian breast; *B*, from abdomen tanned by roentgen-ray exposure, showing many melanin granules; *C*, from normal negro breast, likewise containing a lot of melanin which rather obscures the dopa-positive cells (Laidlaw, Anat. Rec.)

deficiency in neurodermatitis (a form of eczema)—the first such deficiency to be discovered in man

More light is needed on the responsiveness of the living fraction of the epidermis

to influences which affect it through its dead outer shield as well as from the inside within. One factor in keeping the corneal investment in good condition is the outpouring of fatty materials from the sebaceous glands. These prevent water from spreading and inhibit the spread and entry of watery fluids. A duck rises high in the water because water will not mix with or spread on its feathers. If a surface tension reducing substance, aerosol, is added to the water it will pull the water up only its head and neck above water like a periscope on a submarine (see illustration in Duemling's 1941 paper).

A host of influences impinge on the stratum corneum. Some filter through to the living epidermal cells beneath. We think at once of all the kinds of contact dermatitis of war gases, carcinogens, pathogenic microorganisms and viruses, and we should remember the regional diversity of the epidermal sheet and the pits from which the hairs project in which materials can lodge and where the approach to living epidermal cells is most direct.

It is proper, however, briefly to sketch the influence of light. This can be good and bad. That ultraviolet rays in sunlight serve to activate ergosterol and produce vitamin D is well known, but there is some question as to the level in the epidermis at which this excellent result is consummated because these rays penetrate only a short distance. This action may be accompanied by a tanning of the skin. The grip of the endocrines is evidenced in a curious way. The skin of castrate males will burn when thus exposed but it will not tan. However, days after the exposure tan will appear if the individual is injected with testosterone. Without this hormone in these people the stage is set for tanning but they remain pale.

Too much sunlight repeated through the years causes epidermal cancer especially in blonds. Texans pride themselves on being sunburned and continually expose themselves despite warnings. In consequence the frequency of skin cancer in Texas is so great that the U. S. Public Health Service is investigating the matter. It is a fact that ultraviolet light can produce the same malignant transformation of epidermal cells in animals.

Perhaps both vitamin D and cancer production have a common basis in the action of ultraviolet on the sterols for vitamin D is irradiated ergosterol and the steroid hormones which can be carcinogenic can be produced by degradation or synthesis of cholesterol.

In people having a dark complexion and in the colored races the epidermal cells contain a good deal of pigment chiefly melanin (from the Greek black). When seen in sections of the skin melanin is found to be present in tiny granules of uniform size and of yellowish brown color. In brunettes it may be restricted to the basal cells and even in them to the cytoplasm on the distal sides of the nuclei as if protecting the nuclei from harmful solar radiation (Fig. 301). In heavily pigmented skins the pigment extends up into the more superficial layers.

Cells capable of producing melanin are termed melanoblasts and are blackened when treated with dopa oxidase (Technique p. 63). Distribution of dopa-positive cells in the epidermis is represented in figure 302. Whether there exists a second class of epidermal cells remains to be determined. Besides the epidermal melanin is normally found in the reticular body elements of a part of the brain and adrenal medulla. Cells of ectodermal origin are mainly shed. Removal is by desquamation from the skin and by excretion in the kidney and intestines. It is carried in mesenchymatous plasma vesicles called melanosome. For details as to synthesis and excretion of melanin see Jacobson and Hirsch.

1934 This seemingly inert pigment is greatly increased in amount in some highly malignant tumors (melanomas) Frequently ability to produce the pigment is possessed by them and can be demonstrated by the very useful dopa oxidase but the tumors themselves may not be very dark and are therefore called amelanotic melanomas

Much has been written about the types of cells in the epidermis The basal spinous, granular, lucid and corneal cells are regarded as stages in progressive differentiation of a single type The above mentioned melanoblasts may be simply

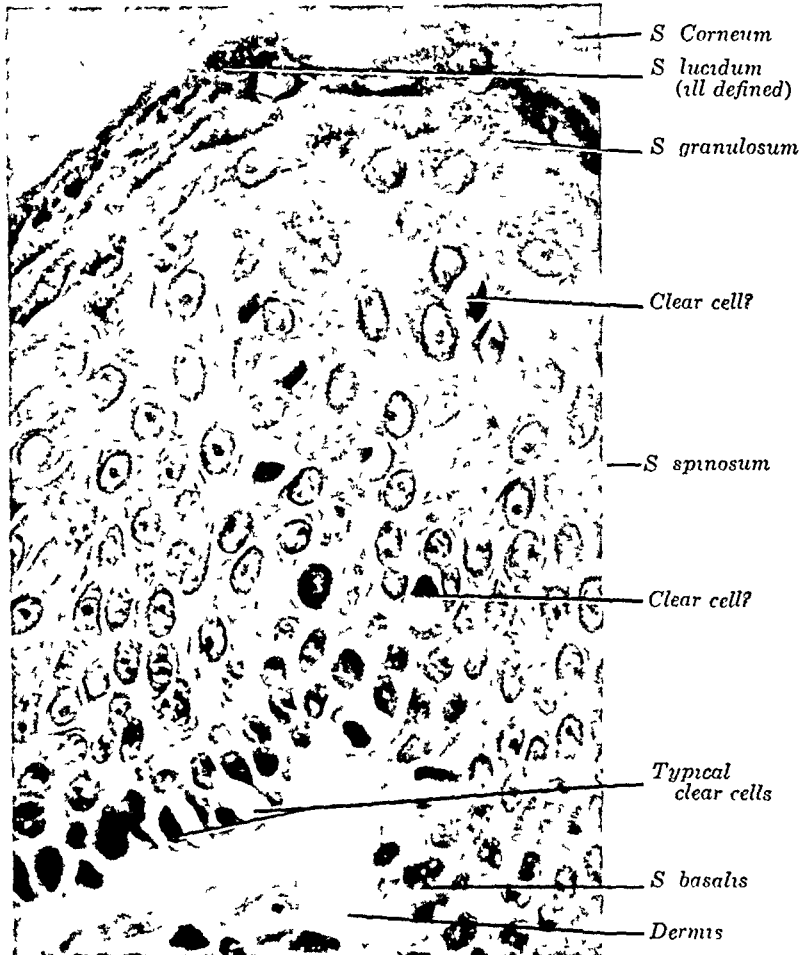
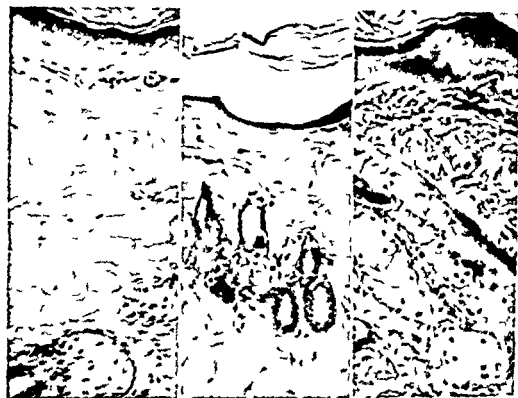


FIG 306—Clear cells of epidermis Biopsy specimen from right cheek in woman aged fifty-seven, having basal cell carcinoma of right forehead Bouin fixation, H and E  $\times 595$  (Barnard Hospital, No 90840, lent by Dr Zola Cooper)

ordinary epidermal cells that for some reason are more active melanin producers than the rest Certain "clear cells" have been described and are illustrated in figure 306 They usually occur near the basement membrane and, perhaps deceptively, look empty Those situated nearer the surface are particularly in question because of possible confusion with mesenchymatous invaders Evidence is unconvincing that typical clear cells are anything more than the usual run of epidermal cells, locally modified Some hold, however, that they are the tactile cells of Merkel and Ranvier, others that they are melanoblasts, still others that they give rise to moles (see review by Ormsby and Montgomery, 1943)



Epidermis is a highly labile and responsive tissue influenced from within as well as from without. It lives on what it gets from the dermis. But the specific requirements of epidermis and its characteristic metabolism will remain obscure until it is systematically studied by itself after removal from the dermis. Endocrines certainly are involved as evidenced by pigmentation of the areola at the nipple induced by pregnancy. See good review of endocrines in relation to dermal life by Seyringhaus (1911). Hooker and Pfeiffer (1913) have noticed that marked atrophy can be experimentally induced in males by estradiol benzoate which is



*Estradiol male*  
Urethra 100x field

*Male ten months*  
53 µg  
Estradiol benzoate  
twice daily

*Male seven months*  
85 µg Estradiol  
benzoate twice daily

FIG. 37. Skin atrophy in rats. Administration of estradiol benzoate in the present causes atrophy of epidermis and sebaceous glands. When testosterone was given to a male rat skin resembles that of untreated animals. Reproduction of Fig. 37 by permission of Hooker and Pfeiffer, courtesy of The University.

relieved by testosterone (Fig. 40). Vitamin A plays some part in epidermal maintenance because it has been discovered that when this vitamin is lacking keratinization spreads in epidermal derivatives and appears in epithelial not only in the skin itself (Wollbach and Howe, 1923, 1924) so much so that vitamin A has been dubbed the "antikeratinizing vitamin." In addition, the discharge from the epidermis gives to the body small amounts of vitamin D, which is a vitamin exclusively affected by sex hormones. Note that the epidermis is a self-regulated apart from dermis which helps to maintain it. It is the dermis that determines the form of the terminal

Before passing to a consideration of dermis it is desirable for students to compare with it at least the stratified epithelia of the tongue (Fig 110), esophagus (Fig 112) urinary bladder (Fig 191) and vagina (Fig 294). Marked differences will be found in pigmentation, keratinization, lamination and other features although all are constructed on the same fundamental plan.

**Dermis.**—This base, upon which the epidermis is built, extends from the epidermal basement membrane to the subcutaneous tissue the beginning of which is not sharply defined especially where the skin is tightly bound down as in the palms of the hands and the soles of the feet.



*Skin, twenty year old white male*



*Skin, eighty-six year old white male*

FIG 308 —Biopsy specimens of young and old antecubital skins. Note less folding in the latter and difference in appearance of dermis. Bouin's fluid and H and E.  $\times 200$  (From Evans, Nielson and Cowdry, courtesy of Anat. Rec.)

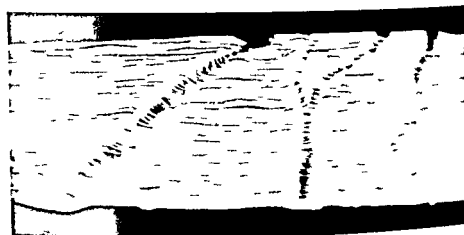
Where the epidermis is thickened and projects as ridges, seldom as papillae or pegs, into the dermis (Fig 301) two parts of the dermis are recognized. The outer

one is the *pars papillaris* shown in figures 302 and 303 and so called because in sections the dermis seems to extend outward into the epidermis as papillae beneath the outward projecting epidermal ridges. The inward limit of the *pars papillaris* is marked by an imaginary line joining the innermost surfaces of the ridges.

Where on the other hand the epidermis is fairly flat with few ridges the *pars papillaris* is inconspicuous, or lacking and the dermis exhibits only the reticularis.



*Epidermis, twenty year old white male*



*Epidermis, eighty six year old white male*

FIG. 299. Reconstructions of epidermises of same specimens as those at page 777.  
303.  $\times 70$  (From Evans, Nielson and Cowdry, courtesy of Anat. Rec.)

Dermis is made up of some components always present and of others present only in certain regions. Among the first are connective tissue fibers. The collagenic ones have great tensile strength which is preserved in the matured leather. The Latin word for leather is *corium*—a term occasionally used for skin. Elastic fibers are also numerous. When the skin of the back of the hand of a student is pulled up between finger and thumb and released it snaps back immediately whereas that of the middle instructor subsides slowly and with decrease in elasticity is reduced. The mesenchymatous cells already described (p. 26) are

present in variable numbers. Phagocytes, which take up melanin, made in the epidermis, are labelled chromatophores because they become colored. The pigment characteristically is present in cytoplasmic masses of irregular size. Comparatively staple mineral and vegetable pigments are pricked through the epidermis into the dermis in tattooing. They, also, are segregated in the macrophages and exhibit a truly remarkable permanence in the body, which, in other respects, is such a changeable structure. We do not know how long the charged macrophages individually live, but they ultimately disintegrate, when the released pigment must be very promptly ingested by other cells of the same sort, for otherwise more of it would be cleaned out *via* the lymphatics and deposited in regional lymph nodes where only traces can be detected. Blood vessels are of course numerous, likewise nerves and lymphatics for the avascular epidermis above must be nourished and the dermis is subject to injury and invasion by microorganisms.

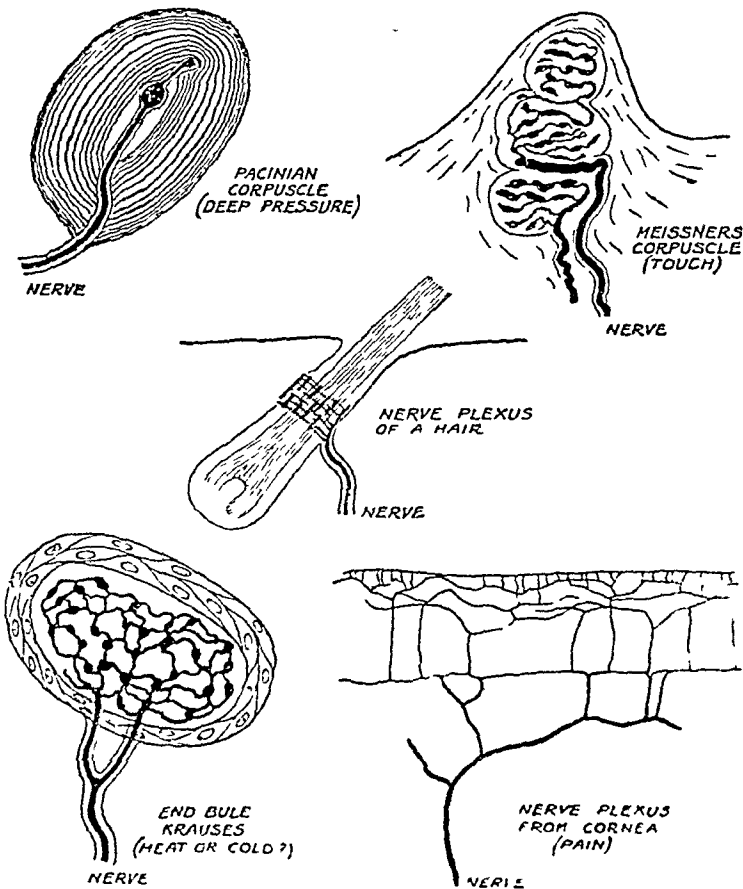


Fig 310 —Cutaneous end-organs and sensations (Starling's Human Physiology; courtesy of J and A Churchill Company, Ltd)

The less constantly occurring constituents are sense organs, hairs and glands. These will be separately discussed later. The distribution of muscle is sharply localized. Striated fibers merge with the dermis of the face (muscles of expression) and with the mucous membrane of the tongue. Smooth fibers are associated with the hairs and are found in a large sheet connected with the scrotal dermis.

In examining sections of the skin remember that as soon as skin is excised the dermis starts to draw itself together—a process increased by fixation—so that folds

already present are exaggerated and new ones may be created. Particularly true in young skins in which the shrinkage averages 36 per cent whereas in the old it is found to be only 12 per cent (Evans, Cowdry and Nielson 1943). See figs 308 and 309. This shrinkage and folding is to be expected from the behavior of exercised arteries in which decreases in length amounting to 40 per cent are reported. Arterial walls would probably make just as good leather as dermis.

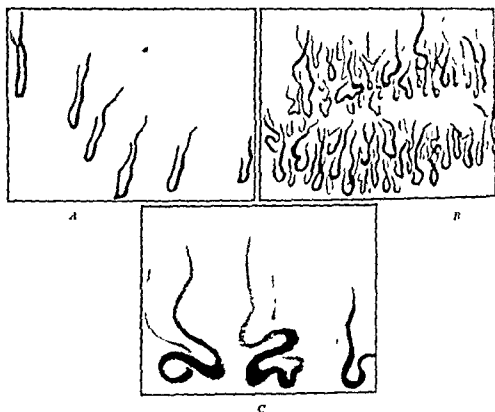


FIG. 311.—Human skin capillaries from finger nail bed examined in the live state. *A* Normal. *B* numerical increase in morbus cruriosus. *C* enlargement in syngamiasis.  $\times 70$ . (Redrawn and modified from Müller, *Die Kapillaren der menschlichen Körperoberfläche*, Stuttgart: J. F. Enke.)

*Nerve fibers*, when present in bundles, are easily identifiable. The endings in muscle have been described. Perhaps naked terminals of nerve fibers in the epidermis and dermis are responsible for pain sensation (fig. 110). Because pain is such a very important signal, Sir Thomas Lewis (1912) took on the subject and it will be studied. Of all the end organs Meissner's corpuscles are the easiest to locate. They are corkscrew-like structures located in dermal papillae of palmar and plantar skin. So infrequent are they in routine specimens from other areas that it is not worthwhile for time pressed students to search for them. Lacunary corpuscles are large, typically laminated, and leap to the eye. They are more deeply situated in the dermis. Consult Weddell (1941) on regional sensibility and Huxley (1941) on correlation between function and histological structure. Literature on the debated influence of hypnosis suggestion on the skin is reviewed by D. A. Smith. The *Utricle* in the dermal papillae can be studied directly when the epidermis is rendered transparent by the addition of a highly refractive oil as was first done

covered by Lombard in 1912. This ability, with an ordinary microscope, to look through the epithelial investment and watch the behavior of the peripheral blood vessels in health and disease and to record the changes by moving pictures, has

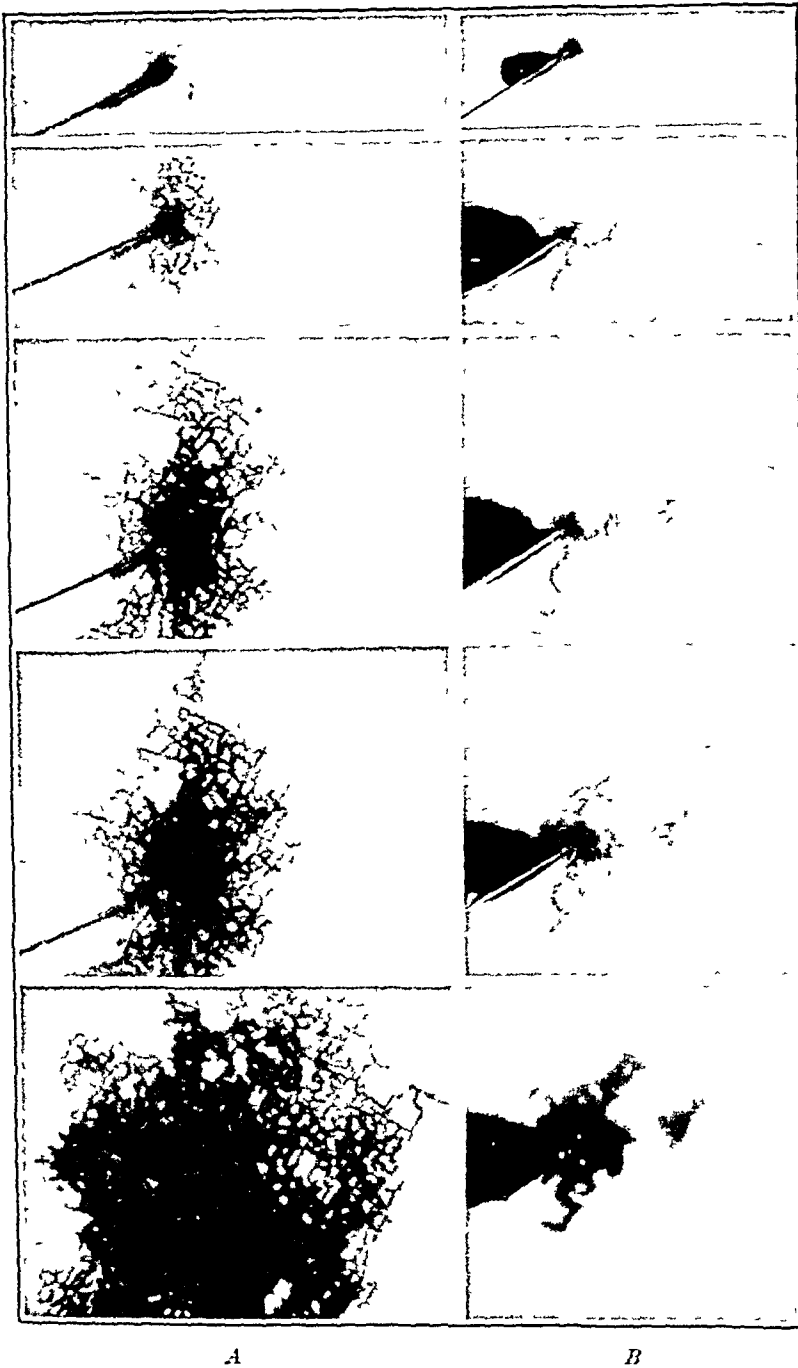
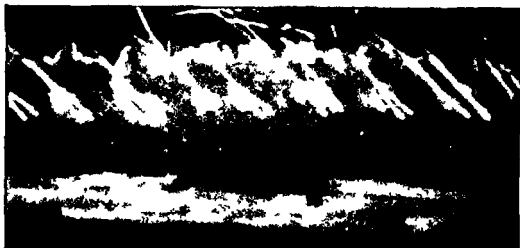


FIG 312—Successive stages in the distribution of dye during injection into the skin of two individuals. Patent blue V in 11 per cent solution was used—0.1 cc. in A and 0.2 cc. in B. The photographs were selected from a moving picture film to show the exact course of events and individual differences. (Hudack and McMaster, J. Exper. Med.)

been productive of significant advances. When the skin is examined directly the capillary loops in the papillae are seen end on. A more favorable view is secured of the vessels in the thin fold of skin covering the base of the finger nail, for here



*Normal skin iron hematoxylin*



*Normal skin ultraviolet fluorescence*



*Ultraviolet fluorescence 15 minutes after application of 0.1 per cent methoxychlor to normal skin*

they stretch out in a distal direction parallel to the surface. Figure 311 gives a faint idea of the range of appearances seen. It is copied from Muller's (1922) Monograph which contains illustrations of a quality that has never since been equalled.

The dermis is a place where emergencies must be met. It is therefore not surprising that *lymphatics* are provided in abundance as a supplementary means of draining away materials in the tissue fluid whose particle size is too large to give entry into the blood stream. When not dilated these vessels may not be readily identifiable. They are shown expanded under the buccal mucous membrane in figures 55 and 56. They can be quickly demonstrated in human subjects by injection of India ink (Fig. 306). McMaster (1942) has thoroughly presented what is known about lymphatic participation in cutaneous phenomena.

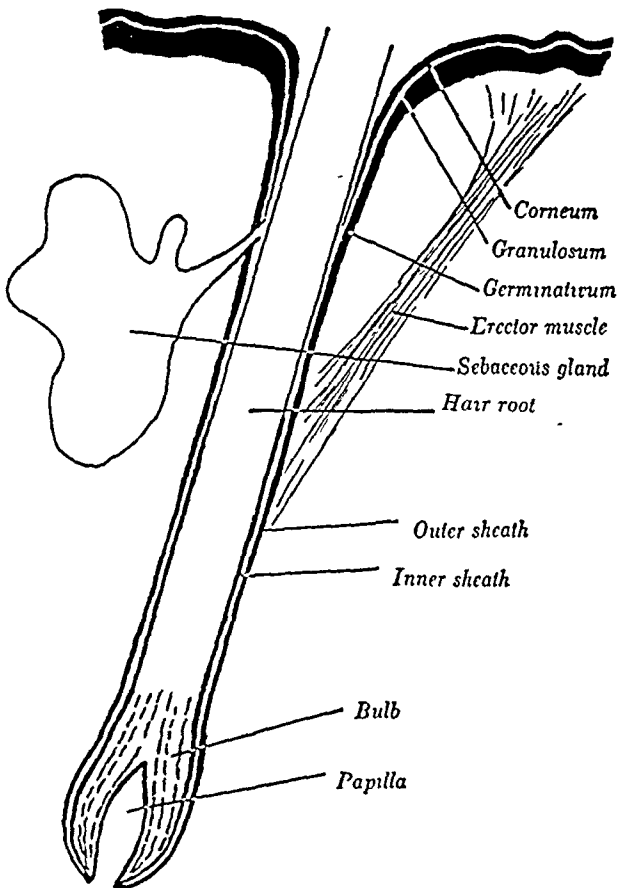


FIG. 314 —Diagram of human hair follicle vertical to the surface of the skin

**Hairs.**—These epidermal appendages are of great interest. They function chiefly as a supplementary protective covering, which, since it retards air currents, also reduces loss of heat. Hairs, with their associated sebaceous glands, are illustrated in figure 314. They are placed at an angle and show a typical fluorescence in ultraviolet light. The sebaceous glands near their roots fluoresce more strongly after application to the epidermis of the carcinogen, methylcholanthrene, which itself is fluorescent.

Structurally each hair consists of a shaft projecting from the surface and of a root encased in a follicle (Fig. 314). The shaft possesses a thin covering of dead,



scale-like epithelial cells comparable with the cornified cell of the general epidermis and a central core composed of other cornified cells which are fibrous elongated, contain distinctive pigment and are interspersed with air. The follicle is a tubular invagination of the epidermis continuous with the root into the end of which a dermal papilla projects and carries blood vessels, nerves and lymphatics for the actively multiplying cells. The stratum corneum can be traced down into the follicle to about the place where the duct of the sebaceous gland enters. The stratum granulosum proceeds a little farther while the germinativum gradually thins to form the external root sheath which is continuous with the bulb at the base of the papilla. The cells of the root immediately capping the papilla are proliferative and correspond to the stratum germinativum. Those farther laterally are divided into several layers the nomenclature of which is difficult to remember and unimportant. The outermost layer is termed the inner root sheath. The root cells gradually cornify as the surface is approached. The inner root sheath is continuous with the cuticle of the shaft. When about one-half the distance from bulb to surface is passed the root is completely cornified all the cells in it having died. The figure also shows the origin of the smooth erector muscle in the dermis and its insertion in the connective tissue which is condensed about the proximal half of the follicle.

The activity of hair follicles like so many other structures is cyclic. A growth period of several weeks is followed by a rest period when the hairs fall out and the next period of growth is begun (see Trotter, 1932-1933). The current idea that hairs continue to grow for days or weeks after death is derived from the fact that exhumed bodies often seem in need of a shave. Some growth is possible and to be expected because it is clear that we all die piecemeal and not at a single instant but the appearances noted at least partly may be due to shrinkage of the dermis causing the hairs to be edged out in the direction of least resistance. Hair receptors are described by Bishop (1911).

The color of hair depends upon 3 factors. Melanin is present as tiny brown granules. When present in high concentration it gives a black appearance. A reddish pigment or pigments occurs more diffusely and depending on amount confers various shades of red themselves often modified by melanin. White hairs are due to the absence of pigment. Exactly what conditions the absence is a mystery. Perhaps hereditary, endocrine factors and dietary are all involved. As much interest attaches to the vitamins and the graying of hair that they have been editorially discussed (J. A. M. A. 118: 302, 1912). That black rats can be turned gray by a diet deficient in pantothenic acid (vitamin B) seems to have been established but to restore hair to its pre-time color by giving this vitamin to patients has not been entirely accomplished.

Hairs also are classified by their size and shape. See Trotter's (1932) description. This is mainly of interest to anthropologists. For us a more dynamic classification of hair in relation to exhumation (Danforth, 1934) is instructive.

1. *Simple or Primary*. The down, wool, lanugo or fuzz hair like that on the forehead is apparently unmodified by sex.
2. *Androgenic*. Hair of this kind can be activated by both male and female hormones. It occurs in the axillary and inguinal regions but the extent of the hairs on the rest of the body varies.
3. *True Secondary*. This category is the beard which is directly controlled by male sex hormone.

As would be anticipated, both masculinizing and feminizing hormones influence the growth of hairs in classes 2 and 3. Ordinary baldness is inherited in the males and skips the females. However, male hormone dominates baldness gene, for those who by inheritance would be bald are not so if castrated early in life. They do not receive the hormonal stimulation which is an incitant to common baldness, in its absence the baldness gene does not operate and their heads are well covered with hair (Hamilton, 1942).

**Sebaceous Glands.**—These glands produce a fatty, oily substance, sebum (L. tallow). They are usually closely related to hair follicles (Figs 312 and 313). Their secretion, poured out on hairs emerging from the pits, conditions and covers the nearby epidermis. In some parts of the body, which are not hairy, sebaceous glands discharge directly onto the surface (glans penis, labia minora, lips, etc.). None are found in the dermis of the palms and soles.

In structure, as well as in development, these glands are inward projecting epidermal appendages lodged in the dermis. The ducts are lined with stratified squamous epithelium continuous externally with the stratified epithelium of the secretory sacs. The cells of the sacs undergo a fatty metamorphosis and those next the lumen are discharged into it, hence the name holocrine (G. *holos*, all, + *krinō*, separate) signifying that all the cell substance, not merely a part of it, is separated out. The testicles and the ovaries belong also in this category since their products are whole cells. By contrast, acinous cells of the pancreas are examples of another large class of merocrine glands (G. *meros*, a part), so named because only a part (the secretion) is thrown out.

Because the products of sebaceous glands are fatty cells, two factors present themselves for consideration. The first is the production of the cells. Scrutiny of sections seldom reveals mitotic figures in the basal cells of the sacs; but a few of them may occasionally be seen in the deeper layers of the epithelial walls of the ducts. Whether a mitotic rhythm exists with peak of cell division at night, as in the epidermis, remains to be determined. The cells evidently shift from the ducts into the sacs to make good those lost as secretion. It is a logical assumption that the new cells are produced in crops in young persons who are subject to acne, for the plugging and infection of the sebaceous glands is periodic as if hormonal stimulation by androgens, or by estrogens, were also periodic. Castrates do not suffer from acne.

Perhaps this stimulation changes the character, as well as the amount, of the fatty metamorphosis. The fatty change involves accumulation in the cytoplasm of fatty droplets, readily stainable with sudan III. The volume of the cells increases moderately, but never reaches that of fat cells, and the droplets do not coalesce as in fat cells. Ignorance persists as to the physiological mechanism of control. Certainly the impulse to produce sebum is insistent. When the openings remain blocked secretion continues and large sebaceous cysts may form. There is marked regional difference in sebaceous glands. All do not develop acne. The meibomian glands of the eyelids show peculiarities.

**Sweat Glands.**—In many particulars these glands differ sharply from sebaceous ones. They are more widely distributed in different regions of the body and are less commonly found in the animal scale. Sweat glands are abundant in mules, donkeys, humans and a few other mammals but students will look in vain for them in most laboratory animals. Their secretion is thin and watery, not cellular, and they discharge on the crests of epidermal ridges, not in hair pits.

In structure sweat glands are of the simple tubular type, not alveolar like the sebaceous ones. The body of each gland is coiled up into a rather compact mass deeply embedded in the dermis. Its epithelial cells are columnar in shape, disposed in a single layer backed by a prominent basement membrane. One often finds in the cytoplasm conspicuous granules which fluoresce brown. Sometimes these are not only of uniform size but double so that they look slightly like bacteria.

A long duct connects the glandular body with the epidermis. As the epidermis is approached the wall increases in thickness to several layers of cells. This duct comes off breaks away from the body locked in the dermis when epidermis is separated from dermis by the acetic acid method (Fig. 301, 1). The lumen of the duct continues through the epidermis in a kind of spiral. It is really a tissue space lined by epidermal cells, mostly keratinized (Fig. 302).

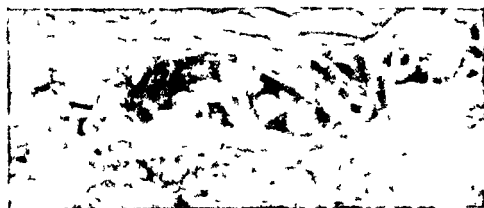


FIG. 311. Sweat gland, 10 per cent formalin fixation, frozen section, fluorescence in ultraviolet light  $\times 140$ . Biopsy specimen from thirty-two year old female. Cells of gland are shown in center of figure. Some cross-sections of it gave golden brown fluorescence of large cytoplasmic granules. Others without them gave bluish gray fluorescence. Most of the fibers about the gland were collagenic and gave a dull white fluorescence but a few elastic fibers gave bright white fluorescence well indicated in the figure. (Photomicrograph by Dr. W. L. S. Jones.)

Sweat glands are quite divergent in their structure in the several regions of the body. The bodies of the glands of Moll, situated at the margins of the eyelid, are irregularly twisted tubes with wide lumina and discharge on the surface, or into the hair pits. The ceruminous glands of the external ear are large deeply placed structures that frequently open, with neighboring sebaceous glands, into hair pits. Sweat glands of the axillary and pubic regions are also of unusual size and extend into the subcutaneous tissue. They have been styled odoriferous glands or Lawrence glands and write content, in x they become activated in the menstrual cycle. Irritation of the ceruminous glands probably felt by other sweat glands but to a less degree.

Sweat glands can pour out a large volume of fluid and the body can lose as much as half of its water as to establish a deficiency which requires correction. A series of papers by Wey and Memmesheimer (1910) supplies keys to the literature. Neumaier et al. (1911) have shown that at the return of sweating is a measure of the return of functional activity of crushed sympathetic nerves in cats.

**Hairs.**—The body of the hair is the visible part and the root the portion embedded in the epidermis. Most of the body is pink because the bed beneath is vascularized. The root consists of stratum germinativum closely attached to

dermis to the underlying bone. But this layer underneath, the lunula, is particularly thick so that the lunula appears whitish partly because the color of the blood is blocked out. The root is similarly based on germinativum which constitutes its matrix. It is by cornification of the cells multiplying in the matrix and beneath the lunula that the nail grows. Its substance shifts distally over the stratum germinativum which remains stationary. To the initiated the appearance of the nails is very revealing as to their owner. Thickening and thinning, transverse and longitudinal ridging, white spots, all have their significance (see Ormsby and Montgomery, 1943)

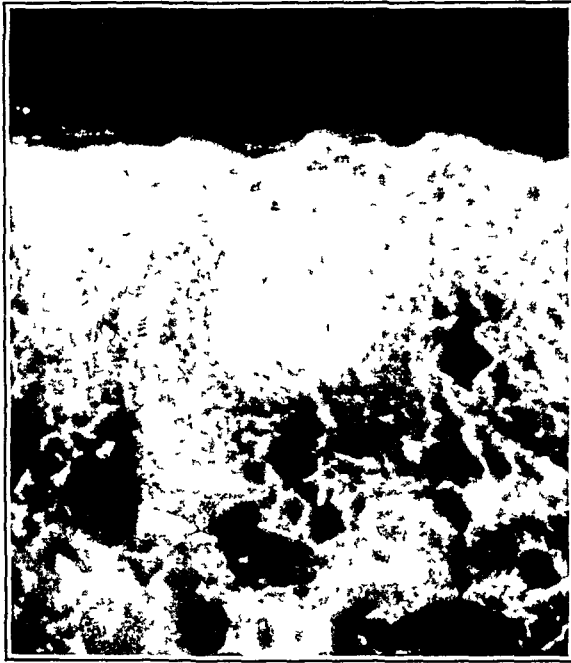


FIG 316—Abdominal skin (of an executed negro, aged thirty-two years), frozen in liquid air, dehydrated *in vacuo* at  $-20^{\circ}\text{C}$ . Six-micron section incinerated and photographed in the dark field. The light reflected from the mineral residue is particularly marked in the epidermal layers. Note that the amount of ash left by the deeper strata is the same as that of the most superficial ones.  $\times 300$  (Photomicrograph by Gordon H. Scott)

### SUMMARY

The skin is a very wonderful mechanism. Slight wounds are continually being repaired. Great strides have been made in our understanding of the factors involved (Arey, 1936). It also renews itself continually. Figure 317 represents the skin of a woman aged one hundred and eleven years. Its excellent condition is impressive.

The protection afforded by the skin is of many kinds. The mechanical advantage of having the body coated with an almost impermeable layer of dead material is evident. The living reactile cells beneath are shielded from pathogenic microorganisms and many gases and water-soluble poisons because the stratum corneum is conditioned by a fatty secretion from the sebaceous glands. Bacteria do not grow well on the surface. Some believe that the epidermis is slightly bactericidal. The absorptive screen afforded by melanin against ultraviolet light has been mentioned. Epidermis and dermis cooperate in protection against mechanical injury and extremes of temperature. Heat is retained by hair and is increased by

vascular engorgement. It is decreased by vasoconstriction and by evaporation of fluid from the sweat glands. When epidermal cells are injured or killed as in a burn (histamine?) is liberated which causes increased capillary permeability so that the area is flooded with fluid (becomes edematous). The fluid is later carried off, particularly by the lymphatics. When the epidermal layer is broken so that extraneous materials enter, the phagocytic cells of the dermis go into action and scar tissue is formed, drawing the edges together and closing the opening.

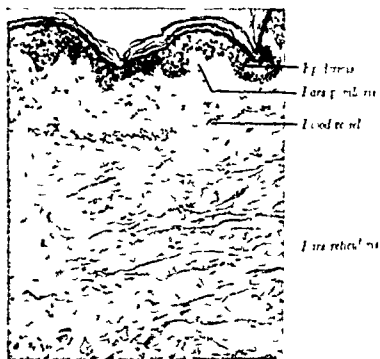


FIG. 317 - Section of skin of woman aged one hundred and eleven years

*Adjustment* - the other equally essential side of the picture for if isolation from the environment were carried to an extreme there would be an end of all physiological activity. The skin cannot afford to be too exclusive. But the information furnished in by the somatic receptors must be carefully chosen for otherwise it would lose point. The irritability of the receptors is heightened for certain kinds of stimuli and depressed for others. The receptors are sensitized, trained to perceive environmental changes that ordinary epidermal cells do not notice and to ignore the unimportant. They are stationed within the epidermis or beneath it and may be equipped with elaborate apparatus for the collection and intensification of the stimuli (eye and ear).

In addition to protecting the internal tissues from the environment and to informing them about what happens on the outside, the epidermis has other functions. It performs as an integral part of the body. The excretory and respiratory functions evident in certain lower forms are rather neglected. It is in the skin that erythema is activated by ultraviolet light. The resulting product (vitamin D) is carried by the blood stream like a hormone to all parts of the body affecting the parathyroid glands. Blood calcium and phosphorus. Opinions are divided as to the particular stratum in which this activation takes place. The skin absorbs light of

sugar present in excess and releases it again when needed. It also takes part in many immunological reactions.

Obviously the epidermis must be maintained by the internal tissues. It receives oxygen, water, nutriment and vitamins by the blood stream, also various hormones. The latter may be divided into two groups, those produced by the thyroid, adrenal, pituitary and perhaps other endocrines which are necessary for its normal function, and the primary sex hormones which excite alterations in the epidermis and its derivatives leading to the development of certain secondary sexual characteristics. The nervous system also helps to regulate the caliber of the cutaneous vessels and the activity of the cutaneous glands. The influence of the emotions on the skin is sometimes startling. The claim that local changes can be produced in the skin by hypnotic suggestion remains to be substantiated. The skin may surpass even the liver in the multiplicity of its functions.

## CHAPTER XVI

### PROSPECT

It is a healthy experience in reviewing the structure of the human body to attempt to formulate the principles on which Nature seems to build. However, no two people will ever view the body in the same light. What impresses one as of consequence may be ignored by another whose background of experience is different. This is but natural for each individual approach is necessarily narrow. It does not mean that either shows lack of judgment. Both may contribute to the whole picture. The obvious danger in trying to take a broad view is the tendency to include dimly seen rules of action concerning which the individual is ill informed. I freely admitted the great complexity of the organism, also the fact that structure and function are inseparable, that a steel needle cannot exist as a structure without showing the properties of sharpness, smoothness, hardness and so on, any more than a cell can possess structure characteristic of life in the absence of its functional expression. Structure is organization of material in space, however large or small. Biochemists and physiologists devote themselves to structural principles just as truly as histologists and pathologists. It might be a relief to medical students if all four would try to express in simple terms the half dozen guiding principles of Nature that they consider most important in moulding the structure of the body. A tendency to become discursive and to mention many more than six must be resisted because in the quest one can so easily let winged fancy roam.

From the histological point of view reliance is placed on water as the essential medium. This appears to be Nature's basic rule of action. It has been so from the beginning of cell life on the globe. Up through the ages we have with each reformation produced watery environments similar to the primordial ocean. Those who perchance read these words do so through films of salt water. They may thus withdraw away with a sigh of relief in which event they find momentary refreshment by increased absorption of atmospheric oxygen through thinly spread salt water in their pulmonary alveoli. Later on they may listen with approval to criticism of the wild thoughts expressed, but they only can do this by using the bodies of salt water in their cochleae. They must even smell and taste the salt water. Nature is conservative and sticks to mechanisms of demonstrated utility.

We are bound on the wheel of water. Without exception all living cells are aquatic and it is by water transport of materials that the body is made a whole. In his fascinating book on the fitness of the environment Henderson (1917) remarks that "It was a surely not chance that led Thales to found philosophy and science with the assertion that water is the origin of all things."

At the beginning of this book reference was made to Claude Bernard's conclusion that "all vital manifestations, however varied they may be, have but one condition, that of presence in a constant conditions of life in the internal environment." J. B. S. Haldane's belief that "No more pregnant sentence was ever framed by a physiologist." Walter Cannon has elaborated this concept in his doctrine of homeostasis. The internal fluid environment and repeatedly refers to it as "the blood stream" but his real meaning applies mainly to the blood stream.

It can hardly be doubted that *provision of different fluid environments makes specialization possible* and can be regarded as a cardinal principle in body building. The tissue fluids of each group of cells, serving in a particular way, are in fact nicely adjusted to their needs. Levelling uniformity, or equality, is not the order of the day. We have seen, that some groups require a tissue fluid served by an abundant blood supply (thyroid, kidney), and others so little blood that they are styled avascular (epidermis, cornea, cartilage); that some are provided with lymphatic drainage on a generous scale (lungs, intestine), while others are alymphatic (bone marrow, brain); that some, near highly permeable sinusoids (liver, spleen), receive materials denied to others supplied by less permeable capillaries (arms, legs); that some need more heat to function properly (ovaries) others less (testicles) and so on almost *ad infinitum*.

That the volumes of the several tissue fluids are adjusted to requirements and held fairly constant is clear. Though a few are measurable in cc and many in  $c \mu$ , all are of considerable size in proportion to their minute cellular inhabitants. That their quality is in many cases also characteristic is attested by numerous observations. We immediately recall the thin watery cerebrospinal fluid, the fluid in the anterior chamber of the eye which typically fails to develop species properties so that it, seemingly alone, is suitable for transplantation of tissue from one species to another, the slippery joint fluids and the fluids stiffened by mineral deposition. A measure of segregation of cellular activities in these environments is every bit as fundamental a feature in architecture as is the joining of activities by integration. More or less permeable partitions and supports giving form and special organization are essential, for, if everything were mixed together, nothing could be accomplished either in the body or a manufacturing plant. Segregation operates on several planes: visible to the naked eye (capsules, membranes, etc.), microscopically visible (the nuclear membranes guarding the integrity of hereditary traits) and ultramicroscopic (adsorption on surfaces of particles, etc.). But isolation of activities cannot be pushed too far. Vital organization depends on a wise blending of separation and integration.

*Differential responsiveness of living cells is the basis of regulation* and perhaps the most fundamental principle in integration. Directional integration, along established pathways, is given by the nervous system. In picking up the message from the external or internal environments advantage is taken of this differential responsiveness. Sensory cells and organs are geared so that they are highly responsive to certain stimuli and ignore the rest. This is clearly the motif in cellular organization and arrangement. Several devices for amplifying the messages have become well established.

The cardiovascular system integrates by a system of broadcasting. Materials are broadcasted to all vascularized parts of the body and there is a parallel wholesale removal of waste. Hormones are emitted from special stations and only cells properly tuned in respond. Again the responsiveness is differential. Very widely used is this principle of differential responsiveness in building the body. It is the spring board of compensatory hyperplasia. Cells in a given organ, such as the liver, increase in number to make good the loss of their neighbors. Mitosis is a basic function, similar in all somatic cells. Yet only the liver cells feel the impulse to divide, and, what is equally interesting, they cease division as soon as the call stops. Superposed on this common responsiveness, possessed by cells of the same type, is a dimly seen variation in responsiveness which may be the basis of rhythms.



Periods of rest alternate with periods of activity. When one makes a list of its activities that are rhythmic it becomes so long as to give the impression that tissues are rhythmic in one way or another. Structural changes occur in rhythms and indeed are inseparable from them. Some of these are clearly discernible others ultramicroscopic while the rhythms themselves are of widely different lengths (cardiac mitotic menstrual hair follicular). If increasing length were expressed in the colors of the visible spectrum from left to right the body would be a weird and wonderful looking structure rivaling a Picasso painting. Rhythms are conditioned by many factors which we may well try to classify. Among them are the character of the task to be accomplished whether a momentary muscular contraction or the building of a complicated structure and the need for rest. Nature has decreed that the time off duty shall not be uniform in all essential relations but shall be measured by the kind of job to be done and the need for relaxation following it. Cells like soldiers marching or laborers rolling a log can together put forth their best efforts when rhythmically synchronized.

In production systems we find that regulation through differential responses is controlled in still another way. As already stated uniformity in all the physical environments would be inconsistent with specialization. But in the same individual citizen cells responding in the same fashion work not only in substantially the same time fluid surroundings but also are equalized. Acinous cells of the pancreas

as a storehouse and is conveniently located in the portal pathway of absorption from the intestines. It takes in large quantities of carbohydrates and proteins. Calcium and other minerals are stored in the bones and teeth by which act at least two objectives are attained. A source of calcium is provided against emergencies and necessary firmness is given to the bony framework of the body. As one classifies the substances needed by the body, it becomes evident that those unlikely to be lacking are not stored, like the oxygen present in the air all about us.

The economic act of storage and the release from storage on demand is one of the means whereby the composition of the blood—and consequently of the superimposed tissue fluid environments—is held fairly stable. But absolute stability is never achieved in any part of a living organism. The single, highly artificial condition in which it obtains is in the state of vitrification, in which every particle is held in place by sudden freezing and maintenance at a low temperature. In this state life is absent but comes back immediately on release of motion by melting. Such immobilization and recovery is only possible for small creatures because speed in vitrification is the essence of success. Returning to our argument, it would be penurious on the part of Nature to set aside more than the storehouses are constructed to hold, and it would be dangerous not to reduce an accumulation in the blood threatening stability by decreasing intake of food and drink and by eliminating excess. The kidney unhesitatingly and promptly casts away surpluses not susceptible of storage. The gastrointestinal tract, the respiratory tract, and even the sweat glands, cooperate.

Economy is further manifest in the regulation of production of materials within the body. Balance is here attained by acceleration and retardation of production, fairly evenly spread as we have seen over citizen cells of each category. In some cases an excess may be more dangerous than a deficiency, as when the endocrines get out of hand in tumors.

Wear and tear in the body is, however, inevitable. Replacement is arranged in accordance with a principle entirely foreign to any man built machine. In a motor car the parts give gradually less satisfactory service until they are discarded and new parts are installed in their places. In the body Nature does not passively wait for depreciation in efficiency of parts to make itself evident. On the contrary, for most living tissues *continuous replacement with maintenance at the peak of performance is attempted*. New cells rise to take the place of those wearing out at a tempo accurately gauged to the individual need in each replaceable category by virtue of the specific responsiveness alluded to. Recently it has been discovered that there is a brisk turnover in intracellular fat. This material is not permitted to rest indefinitely in fat cells. By some means, difficult to envisage, before fat just stored is utilized, other fat that has been in safe keeping longer is employed so that Nature qualifies as a good housewife. It is not unlikely that other chemical substances are similarly replaced in an orderly manner so there is no time for them to deteriorate. Marking them, by the introduction of tagged atoms, and tracing them through the body will probably clarify the problem.

Equally instructive are the bodily constituents which are replaced little if at all. Let us consider cells first and fibers next. Among the former nerve cells are not replaceable, at any rate after one year of age, and the replaceability of cardiac muscle cells is so inadequate (if it occurs) as to be negligible in our search for architectural guiding principles. Satisfactory performance is assured for many years by the numerical reserve, for, when some cells no longer can continue, others are able

to take over by working a mite harder. It is conceivable that such long lived cells are so constructed that their own substance is itself gradually replaced—a phenomenon perhaps not so marked nor as necessary, in cells whose life span is 20 times 1000 times shorter (neutrophils)—but this is pure speculation.

Fibrous components both collagenic and elastic age as colloids in the body slowly. In the former Nature long ago perfected structures which are plastic and withstand tensile strain. Millions of years later mankind discovered that dermal collagenic fibers can be removed from the body and made to serve as leather—a material not surpassed by any artificial plastics. The elastic fibers are an equal rubber. In tissue fluid environments this material probably retains its remarkable elasticity much longer than does prepared plant rubber, or mixtures of it with rubber with artificial rubber in man built machines. It is not beyond the bounds of possibility that traces of animal elastin might prove helpful in making a good grade of artificial rubber. However this may be in introducing collagen and elastin Nature certainly hit on two very durable classes of material which can be formed throughout life despite the fact that a means has not been found satisfactorily to remove the old fibers and to replace them with new ones.

*Principle of safety mechanisms is a principle that makes for survival.* Nature supplies more than is ordinarily needed. Consequently survival is possible after removal of sizeable fractions of the principal organs.  $\frac{1}{4}$  of adrenal cortex,  $\frac{1}{2}$  of kidneys,  $\frac{1}{2}$  liver and  $\frac{1}{2}$  thyroid and pancreas. These volumetric factors of safety actually result from the multiplication of mechanisms. They depend in other words on the keeping in service of more cells and other units than are absolutely necessary by lightly spreading the labor over them so that it amounts to but a small part of the total but they can do in an emergency. One observes that resort is not made to the growth of bigger and better cells because limitations in effective cellular and organismal sizes are strictly drawn. On a slightly different basis is the duplication of mechanisms. Pairs of many structures are provided. It is not uncommon for important organs to draw blood from several sources. If one fails the others can carry the load. We have noticed that some enzymes and hormones are produced not only in a single organ but also in others sometimes far distant, but reliance on these other producers is but a slender need. The caliber of blood vessels is subject to hormonal as well as to nervous control as a car can presumably be stopped by both mechanical and hydraulic brakes. Factors of safety, or physiological reserves as they are called of one sort or another seem to be everywhere in the body.

Barriers of many kinds are interposed between the outside world and the living tissues within. All the cells exposed to the air, not protected by water are dead.

While we are in life we are in death' is a true saying. The epidermis is a protection. So also are the sphincters that guard the apertures and the cilia that act as sweepers. Cells capable of producing antibodies make for safety when foreign materials pass the defences. Special groups of cells phagocytize and destroy invading organisms.

The sensation of pain is implanted as an invaluable warning signal. The most insidious and lethal diseases are those not heralded by pain.

*In summary* it can be said that Nature the Master Builder constructs a temporary edifice in accordance with definite principles of value proved through millions of years of which but a few have been sketched leaving the more biologically and physical ones to those better qualified to discuss them. The ways of Nature are not simple. Nothing is more conducive to loss of face than to ignore the details.

that "Nature moves in mysterious ways her wonders to perform." The deeper we dig in any problem, however simple it first may appear, the more complex it gets. Complete understanding of any vital property is quite beyond us. Constant change in adaptation to myriad influences everywhere is manifest. A single part of the whole is never structurally the same at two intervals in time, and the body is so much a unit that the statement is probably justified that if we knew *all* about any part our knowledge of the whole would be complete. Wise physicians acquire increasing respect for the efficiency of natural adjustments and learn to help Nature help herself interfering radically only when she is obviously up against it. They know that the noblest study of man is man and that had our forebears more closely examined the workings of Nature in man they would not have taken so long to invent the principles of the hinge and the lever to say nothing of how electricity is harnessed in muscular contraction first observed by Galvani. And the end is assuredly not yet.

It is abundantly clear that to attain great ends Nature has been daring. Some developments, no longer of service, remain as hazards (appendix, Meckel's diverticulum, etc.) Heredity can be a curse, though it is usually a blessing for on it evolution depends. It is risky to have the alimentary tract cross the respiratory one, because materials passing along it can occasionally enter the larynx and even lodge in the lungs. For urine in males to be conducted out through the prostate is a great handicap to elderly men in whom hypertrophy of this organ is so frequent as to be considered normal. Progressive dominance of the brain leads to much that is worthwhile, but it is not without danger. A phylogenetically new achievement is the invention of supplementary drainage of the all important tissue fluid environments by the lymphatics which surgeons must destroy when they strive to limit the spread of cancer. Another is the utilization of substances as valuable sex hormones, which are chemically akin to carcinogens and, on occasion, can promote the disastrous malignant transformation of cells.

# BIBLIOGRAPHY

## A

- ALLEN A F and ALLEN B C 1942 Anat Rec 82 211-231
- ALLEN B C 1941 J A M A 117 527-533
- ALLEN B C 1927 Contrib to Embryol (Carnegie Inst) 19 (98) 1-44
- 1930 Allen's Sex and Internal Secretions Baltimore Williams & Wilkins 931 pp
- ALLEN B C, HISAW F I and GARDNER, W A 1930 Allen, Danforth and Deery's Sex and Internal Secretions Baltimore Williams & Wilkins 1346 pp
- ALLEN F M 1922 J Metab Res, 1 1-41 75-95
- ALLEN W C 1929 The Mechanics of the Digestive Tract New York Paul B Hoeber Inc 418 pp
- 1937 Quart Rev Biol 12 152-164
- ALLEN W C and BATHURST A 1933 34 Ztschr f Zell u mikr Anat 20 143 205 469 572
- ANDERSON J 1911 Anat Rec 13 269 272
- 1917 Cowdry's Special Cytology 3 1211-1301
- 1918 Physiol Rev 16 327-406
- 1919 Developmental Anatomy Philadelphia W B Saunders Company 612 pp
- 1921 Anat Rec 81 21-33
- 1922 Quart Bull Northwestern Univ Med Sch 16 100 104
- 1923 1937 (See Arey 1936)
- ANDERSON M J and MONZINGO F L 1935 Anat Rec 64 9 27
- 1936 Dutch med Wehnschr, 30 54-56
- 1937 Am J Anat 37 159 193

- BISHOP, G H 1943 *J Neurophysiol*, 6, 361-362  
 BITTNER J J 1942 *Cancer Res*, 2, 710-721  
 BLOOM, W 1927 *Arch Path and Lab Med*, 3, 608-628  
 BODIAN, D 1943 *J. A. M. A.*, 121, 662-664  
 BOEKE, J 1932 *Penfield's Cytology and Cellular Pathology of the Nervous System*, 1, 241-316  
 BOHM, E, GERNANDT, B, and HOLMGREN, H 1941 *Acta med Scand*, 106, 579-598  
 BOLING, L R 1935 *Arch Otolaryngol*, 22, 689-724.  
 ——— 1942 *Anat Rec*, 82, 25-37.  
 BOURNE, G 1939 *Proc Physiol Soc* (Jan 14), *J Physiol.*, 95, 12-13  
 ——— 1942 (Editor) *Cytology and Cell Physiology*, Oxford, 296 pp  
 BOWIE, D J, and VINEBERG, A M 1935 *Quart J Exp Phys.*, 25, 247-257  
 BOYDEN, E A 1929 *Proc Soc Exp Biol and Med*, 27, 86-87  
 BOYS, F 1942 *Surgery*, 11, 118-168  
 BRAUS, H 1924 *Anatomie des Menschen*, Berlin, Julius Springer, 697 pp.  
 BREMER, J L 1944 *Histology* (Lewis and Stöhr), 6th ed, rewritten by Harold L Weatherford, Ph D, Philadelphia, P Blakiston's Son & Co, 580 pp (5th ed)  
 BUCCIANTE, L, and LURIA, S 1934 *Arch ital anat e embriol*, 33, 110-187.  
 BUNTING, C H 1932 *Cowdry's Special Cytology*, 2, 683-707  
 ——— 1938 *Downey's Hematology*, 1, 161-177  
 BURCH, G E 1940 *Arch Int Med*, 65, 477-498  
 BUTOMO, W 1927 *Arch f Gynäk*, 131, 306-324

## C

- CANNON, W B 1930 Chapter X, *Cowdry's Human Biology*, New York, Paul B Hoeber, Inc, 612 pp  
 ——— 1932 *The Wisdom of the Body* New York, W. W Norton & Co, Inc, 312 pp  
 CAPPELL, D F 1929 *J Path. and Bacteriol*, 32, 595-707.  
 CAREY, E J 1921a *Anat Rec*, 21, 189-215  
 ——— 1921b *Am J Physiol*, 58, 182-194  
 ——— 1936a *Am J Anat*, 59, 89-122  
 ——— 1936b *Anat Rec*, 64, 327-341  
 ——— 1942a *Am J Anat*, 70, 119-133  
 ——— 1942b *Am J Path*, 18, 237-289  
 CARLSON, A J 1942 *Cowdry's Problems of Ageing*, Baltimore, Williams & Wilkins, 936 pp  
 CARREL, A 1924 *J A M. A.*, 82, 255-258  
 CARREL, A, and EBELING, A H. 1926 *J Exp Med*, 44, 261-305  
 CARRUTHERS, C, and SUNTZEFF, V 1943 *Cancer Research*, 3, 744-748  
 CASTLEMAN, B, and MALLORY, T B 1935 *Am. J Path*, 11, 1-72  
 CHAMBERS, R, and ZWEIFACH, B W 1940 *J Cell and Comp Physiol*, 15, 255-272  
 CHILLINGWORTH, F P, HEALY, J C, and HASKINS, F E 1933-34 *J Lab and Clin Med*, 19, 486-494  
 CHRISTENSEN, K 1940 *J Dent Res*, 19, 227-242  
 CLARA, M 1930 *Ztschr f mikr-anat. Forsch*, 20, 584-607  
 ——— 1940 *Ibid*, 47, 183-246  
 CLARK, E R, and CLARK, E L 1932 *Am J Anat*, 49, 441-474  
 ——— 1934 *Ibid*, 54, 229-286  
 ——— 1934a *Ibid*, 55, 407-467  
 ——— 1935 *Ibid*, 57, 385-438  
 ——— 1936 *Ibid*, 59, 123-173  
 ——— 1940 *Ibid*, 67, 255-285  
 ——— 1942 *Ibid*, 70, 167-200  
 CIMFRIKO, D R, and PONDER, E 1934 *Quart J Physiol*, 24, 289-294.  
 CIOAKE, P C, LEARMOUTH, J R. and BARRINGTON, F J F 1931 *Proc Roy Soc Med* 25 (1), 517-561.  
 COGGESHALL, H C, WARREN, C F, and BAUER, W 1940 *Anat Rec*, 77, 129-144  
 COHEN, S M 1940-41 *Guy's Hospital Rep*, 20, 201-216  
 COOK, W I. 1927 *J Roy. Micr Soc*, 47, 29-39  
 COOPER, Z K, and SCHIFF, A 1938 *Proc Soc Exp Biol and Med*, 39, 323  
 COPSLIP, G W 1920 *Contrib to Embryol* (Carnegie Inst), 9, 87-93.  
 ——— 1928 *Am J Physiol*, 86, 74-81  
 ——— 1932 *Cowdry's Special Cytology*, 3, 1565-1607.  
 ——— 1933 *Medicine*, 12, 61-82  
 COVILL, W P. 1928 *Anat Rec*, 40, 213-223

- COWLEY W P and SCOTT C H 1928 *Anat Rec* 38, 377-396  
 COWLEY L V 1918 *Contrib to Embryol (Carnegie Inst)* 8 (25) 39-100  
 ———— 1922 (Editor) *Special Cytology* New York Paul B Hoeber Inc 168 pp  
 ———— 1933 (Editor) *Arteriosclerosis* New York The Macmillan Company 417 pp  
 ———— 1942 (Editor) *Problems of Aging* Baltimore Williams & Wilkins Company 136 pp  
 ———— 1943 *Microscopic Technique* Baltimore Williams & Wilkins Company 277 pp  
 COWLEY L V and DANKS W B C 1933 *Parasitology* 25 1-63  
 COWLEY L V and KITCHEN S F 1930 *Am J Hyg* 11 227-299  
 COWLEY L V and SCOTT G H 1936 *Arch Path* 22 1-23  
 ———— 1938 *Ann dell'Inst Pasteur Tunis* 17 233-242  
 CRAMER W 1928 *Fever Heat Regulation Climate and the Thyroid Adrenal Apparatus* London Longmans Green & Co 153 pp  
 CRAWFORD A I 1922 *J Anat* 56 98-106  
 CRAWFORD A I and CHAMBERLAIN H A 1938 *Am J Physiol* 124 398-401  
 CRILE W O, HAHN P F, BALE W R, and BALFOUR W M 1941 *Am J Med Sci* 221 1-107  
 CRILE H H 1939 *Arch Surg*, 22 506-523  
 CUTHBERT M 1918 *Am J Anat* 24 339-394  
 CUTLER HARRY 1942 *Intitutory Body Hypothalamus and Parasympathetic Nervous System* Springfield Ill Charles C Thomas 234 pp

## D

- DANFORTH C H 1939 *Physiol Rev* 19 91-111  
 DANKS W B C 1934 *Biogenesis Cytology and Cell Physiology* Oxford Clarendon Press 447 pp  
 DANKS W B C 1934 *Anat Rec* 34 208-217  
 DANKS W B C 1935 *Cowley's Special Cytology* 3 1369-1407  
 DANKS W B C 1938 *Am J* 1931 *Arch exp Zell* 16 167-186  
 DANKS W B C 1941 *Anat Rec* 79 417-433  
 ———— 1942 *Ibid* 84 12-13  
 DARRICH 1940 *Vertebrate Photoreceptors* New York The Macmillan Company 154 pp  
 DARRICH 1941 *Anat Rec* 3 475-497  
 DARRICH 1942 *Proc Soc Exp Biol and Med* 38 536-538  
 DARRICH 1943 *A J* 1941 *Anat Rec* 80 211-217  
 DARRICH 1944 *W F* 1931-32 *J Anat* 66 610-617  
 DARRICH 1945 *Handbook of Hematology* (4 volumes) New York Paul B Hoeber 1000 pp  
 DARRICH 1946 *Am J* 1931 *J Exp Med* 53 143-150  
 DARRICH 1947 *J M* 1941 *Lymphatics Lymph and Lymphoid Tissue* 100 pp  
 DARRICH 1948 *Arch Dermat and Syph* 43 264-280  
 DARRICH 1949 *Life and Bodily Changes* New York Columbia Univ Press 100 pp  
 DARRICH 1950 *Ibid* 1927 *Anat Rec* 34 313-329

## E

- EASTMAN 1941 *and FLOREY H W* 1940 *Brit J Exp Path.* 21 1-10  
 EBERHART 1941 *Handbuch der Anatomie des Menschen* 7 1-3  
 EDWARDS 1941 *Am Heart J* 19 335-351  
 EDWARDS 1942 *J Exp Med* 76 335-351  
 EGGLE 1941 *Problems of Aging* 475-491  
 EVANS H M 1941 *Mem Univ California* 9 (1) 1-10  
 EVANS H M 1942 *Science* 39 413-414  
 EVANS R C 1941 *Ibid* 1943 *Anat Rec* 86 1-10  
 EVERETT N R 1941 *Ibid* 1922 49-91

## F

- FIGGE, F H J 1942 Bull School of Med Univ Maryland, 26, 165-176  
 FLEXNER, L B, and GELLHORN, A 1942 Am J Obst and Gynec, 43, 965-974  
 FLOREY, H, CARLETON, H M, and WELLS, A G 1932 Brit J. Exp Path, 13, 269-284  
 FOOTE, J J, and GRAFFLIN A L 1942 Am J Anat, 70, 1-20  
 FORSGREN, E 1929 J Morph, 47, 519-529  
 ——— 1931 Acta med Scandin, 76, 285-315  
 FRIEDENWALD, J 1942 Cowdry's Problems of Ageing, 535-555.  
 FUKUI, N 1923 Japan Med World, 3, 27-28, 160-163  
 FULTON, J F 1926 Muscular Contraction, Baltimore, Williams & Wilkins Company, 644 pp

## G

- GAGE, S H, and FISH, P A 1924-25 Am J Anat, 34, 1-86  
 GALLOWAY, R J M. 1936. Am J Path, 12, 333-340  
 GAMBLE, J L 1937 Bull Johns Hopkins Hosp, 51, 151-173  
 GARDNER, E D 1942 Anat Rec, 83, 401-419  
 GENTILE, R J, SKINNER, H L, and ASHBURN, L L 1941 Surgery, 10, 793-810.  
 GILLMAN, J 1942 South African J Med Sci, 7, 144-159  
 GIROUD, A, and LEBLOND, C P 1934 Arch d'anat micr, 30, 105-129.  
 GLEES, P 1943 J Anat, 77, 153-159  
 GLICK, D 1934 Ztschr f physiol Chem, 226, 186-191  
 GLOMSET, D J 1941 Proc Inst Med Chicago, 13, 398-406  
 GOERTTLER, K. 1931 Morph Jahrb, 65, 45-128  
 ——— 1932 Ibid, 69, 329  
 GOLDMANN, E E 1909 Beitr z klin Chir, 64, 192-265  
 GOMORI, G 1941 Am J Path, 17, 395-406  
 GOORMAGHTIGH, N 1922 Le cortex surrénal humain dans les plaies de l'abdomen et aux périodes intéressantes de la vie sexuelle, Thèse, Université de Gand, 90 pp  
 GOSS, C M 1940 Anat Rec, 76, 19-27  
 GRAAM, D G 1942 J Lab and Clin Med, 27, 448-459  
 GRAFFLIN, A L 1939 Anat Rec, 75, 27-38  
 ——— 1940 J Morph and Physiol, 67, 455-470  
 ——— 1940 Anat Rec, 78, 204-214  
 ——— 1942 Am J Anat, 71, 43-64  
 ——— 1942 Ibid, 70, 399-431  
 GRAHAM, E A 1926 Am J Med Sci, 172, 625-643  
 GRANT, R T, and BLAND, E F 1930 Heart, 15, 385-411  
 GREGG, A 1940 J A M A., 114, 1139-1141  
 GREENE, H S N 1938 Science, 88, 357-358  
 GROLLMAN, A 1936 The Adrenals, Baltimore, Williams & Wilkins, 410 pp.  
 GRUBER, C M 1933 Physiol Rev, 13, 497-609  
 GUILD, S R 1927 Am J Anat, 39, 57-81  
 ——— 1942 Cowdry's Problems of Ageing, 556-566

## H

- HACKFL, W M 1928 Virchows Arch f path Anat, 266, 630-639  
 HAGGQVIST, G 1929 Acta chir Scandin, 65, 180-196  
 HALSTED, W S 1919 The Operative Story of Goitre, Baltimore, Johns Hopkins Press, 254 pp  
 HAN, A W. 1932 Cowdry's Special Cytology, 979-1052  
 HAN, A W, and BALDWIN, K W. 1941 Anat Rec, 81, 363-379  
 HAN, A W, *et al* 1940 Am J Path 16, 277-286  
 HAN, A, and PORTONDO, B C 1933 Arch Path, 16, 1-14  
 HAMILTON, J B 1942 Am J Anat, 71, 451-487  
 HAMILTON, J B, SOLFY, M H, and EICHORN, K B 1940 Univ Calif Publ Pharm, 1, 339-367  
 HAMPEL, H 1925 Ztschr f mikr-anat Forsch, 2, 506-535  
 HARPIS, H A 1931 Brit J Radiol, 4, 561-610  
 HARTMAN, C G. 1932 Allen's Sex and Internal Secretions, pp 637-715  
 ——— 1939 Ibid pp 630-719  
 HARTMAN, F A 1942 Endocrinology, 30, 861-869  
 HARVEY, B C H, and BEN-LEY, R R 1912 Biol Bull, 23, 225-249  
 HASS, G M 1942 Arch Path, 34, 807-819  
 ——— 1943 Ibid, 35, 275-284



- HEINER K P, POLK D and WHITE H L. 1913. *Am J Physiol* 139 543-549  
 HELLER R I. 1911. *Surg Gynec and Obst* 74 1119-1127  
 HENLOCK C J. 1922a. *Introduction to Neurology* Philadelphia W B Saunders Company  
 499 pp  
 HERRIN P T. 1908. *Quart J Exp Physiol* 1 121-160  
 HERTZMAN A B. 1912. *Ann. Rev Physiol* 4 187-214  
 HERRING A J. 1938. *Am J Path* 14 593-603  
 HESS M. 1908. *Virchows Arch f path Anat* 261 225-232  
 HEYER H I and HERTON R W. 1941. *Am J Clin Path* 11 819-827  
 HILDING A. 1932. *Arch Otolaryngol* 16 9-18  
 HILL C J. 1908. *Phil Trans Roy Soc Series B* 216 333-388  
 HINES H M. 1912. *J A M A* 120 515-517  
 HODER N. 1931. *Am J Anat* 48 139-197  
 HODGE M J. 1937. *Anat Rec* 67 21-533  
 HODMAN J. 1915. *J Med Res* 28 1-31  
 HOFFER, C W and PRUITT C A. 1943. *Endocrinology* 32 69-76  
 HOLLING R C. 1933. *The Tides of Life* New York, W W Norton 352 pp  
 HOWELL W H. 1900-91. *J Morphol.* 4 117-129  
 HUBBARD S S and McMASTER P D. 1933. *J Exp Med* 57 731-774  
 HUGGINS C and BLOCKSON B H Jr. 1936. *J Exp Med* 64 253-274  
 HUGGINS C, BLOCKSON B H Jr and NOONAN W J. 1936. *Am J Physiol* 115 313-341  
 HUGGINS C, McLAUGHLIN J and WILCE E. 1910. *Anat Rec* 76 309-317  
 HUGHES C STERN, R I Jr and HODGES C V. 1911. *Arch Surg* 43 209-223  
 HUGHES W and CROWT S J. 1931. *J A M A* 96 2027-2028  
 HUMPHREY R L. 1936. *J Exp Zool* 73 1-21

## I

- IMBIA W J and LAWRENTIEW B J. 1932. *Ztschr f mikr anat Forsch* 30 550-542  
 INGLETON C and DANILLIAN A C. 1927. *Am. J Med Sci*, 174 70-87  
 JAY A C. 1914. *Am J Obst and Gynec* 44 962-963  
 1912. *Cowdry's Problems of Ageing* 24-301

## J

- JAY A C. 1916. *Am J Anat* 19 303-332  
 JAY A C and KLINCK G H. 1934. *Arch Path* 17 141-151  
 JAY A C. 1939. *J Path and Bact* 49 1-19  
 JAY A C. 1940. *Int J Exp Path* 23 272-276  
 JAY A C. 1941. *Am J Anat* 56 217-277 279-303  
 JAY A C. 1942. *Ibid* 14 235-250  
 JAY A C. 1943. *Anat Rec* 59 187-199  
 JAY A C. 1944. *Ibid* 22 281-292  
 JAY A C and WILANDER O. 1937. *Ztschr f mikr anat Forsch* 42 277-291  
 JAY A C. 1941. *Am J Anat* 56 217-277 279-303  
 JAY A C. 1942. *Am J Anat* 56 217-277 279-303  
 JAY A C. 1943. *Am J Anat* 56 217-277 279-303  
 JAY A C. 1944. *Am J Anat* 56 217-277 279-303

## K

- KABAT E. 1941. *Am J Path* 17 303-318  
 KALLIOTIS A. 1942. *J Exp Med* 76 307-316  
 KERRIN C. 1908. *The Engines of the Human Body* Philadelphia J B Lippincott  
 KENT C. 1912. *Anat Rec* 75 275-287  
 KENTON A. 1913. *Am J Path* 9 317-368  
 KERCHER A. 1914. *Am J Path* 10 317-368  
 KEY J A. 1915. *Arch Surg* 11 254-303  
 KEY J A. 1916. *Arch Surg* 12 254-303  
 KEY J A. 1917. *Arch Surg* 13 254-303  
 KEY J A. 1918. *Arch Surg* 14 254-303  
 KEY J A. 1919. *Arch Surg* 15 254-303  
 KEY J A. 1920. *Arch Surg* 16 254-303  
 KEY J A. 1921. *Arch Surg* 17 254-303  
 KEY J A. 1922. *Arch Surg* 18 254-303  
 KEY J A. 1923. *Arch Surg* 19 254-303  
 KEY J A. 1924. *Arch Surg* 20 254-303  
 KEY J A. 1925. *Arch Surg* 21 254-303  
 KEY J A. 1926. *Arch Surg* 22 254-303  
 KEY J A. 1927. *Arch Surg* 23 254-303  
 KEY J A. 1928. *Arch Surg* 24 254-303  
 KEY J A. 1929. *Arch Surg* 25 254-303  
 KEY J A. 1930. *Arch Surg* 26 254-303  
 KEY J A. 1931. *Arch Surg* 27 254-303  
 KEY J A. 1932. *Arch Surg* 28 254-303  
 KEY J A. 1933. *Arch Surg* 29 254-303  
 KEY J A. 1934. *Arch Surg* 30 254-303  
 KEY J A. 1935. *Arch Surg* 31 254-303  
 KEY J A. 1936. *Arch Surg* 32 254-303  
 KEY J A. 1937. *Arch Surg* 33 254-303  
 KEY J A. 1938. *Arch Surg* 34 254-303  
 KEY J A. 1939. *Arch Surg* 35 254-303  
 KEY J A. 1940. *Arch Surg* 36 254-303  
 KEY J A. 1941. *Arch Surg* 37 254-303  
 KEY J A. 1942. *Arch Surg* 38 254-303  
 KEY J A. 1943. *Arch Surg* 39 254-303  
 KEY J A. 1944. *Arch Surg* 40 254-303  
 KEY J A. 1945. *Arch Surg* 41 254-303  
 KEY J A. 1946. *Arch Surg* 42 254-303  
 KEY J A. 1947. *Arch Surg* 43 254-303  
 KEY J A. 1948. *Arch Surg* 44 254-303  
 KEY J A. 1949. *Arch Surg* 45 254-303  
 KEY J A. 1950. *Arch Surg* 46 254-303  
 KEY J A. 1951. *Arch Surg* 47 254-303  
 KEY J A. 1952. *Arch Surg* 48 254-303  
 KEY J A. 1953. *Arch Surg* 49 254-303  
 KEY J A. 1954. *Arch Surg* 50 254-303  
 KEY J A. 1955. *Arch Surg* 51 254-303  
 KEY J A. 1956. *Arch Surg* 52 254-303  
 KEY J A. 1957. *Arch Surg* 53 254-303  
 KEY J A. 1958. *Arch Surg* 54 254-303  
 KEY J A. 1959. *Arch Surg* 55 254-303  
 KEY J A. 1960. *Arch Surg* 56 254-303  
 KEY J A. 1961. *Arch Surg* 57 254-303  
 KEY J A. 1962. *Arch Surg* 58 254-303  
 KEY J A. 1963. *Arch Surg* 59 254-303  
 KEY J A. 1964. *Arch Surg* 60 254-303  
 KEY J A. 1965. *Arch Surg* 61 254-303  
 KEY J A. 1966. *Arch Surg* 62 254-303  
 KEY J A. 1967. *Arch Surg* 63 254-303  
 KEY J A. 1968. *Arch Surg* 64 254-303  
 KEY J A. 1969. *Arch Surg* 65 254-303  
 KEY J A. 1970. *Arch Surg* 66 254-303  
 KEY J A. 1971. *Arch Surg* 67 254-303  
 KEY J A. 1972. *Arch Surg* 68 254-303  
 KEY J A. 1973. *Arch Surg* 69 254-303  
 KEY J A. 1974. *Arch Surg* 70 254-303  
 KEY J A. 1975. *Arch Surg* 71 254-303  
 KEY J A. 1976. *Arch Surg* 72 254-303  
 KEY J A. 1977. *Arch Surg* 73 254-303  
 KEY J A. 1978. *Arch Surg* 74 254-303  
 KEY J A. 1979. *Arch Surg* 75 254-303  
 KEY J A. 1980. *Arch Surg* 76 254-303  
 KEY J A. 1981. *Arch Surg* 77 254-303  
 KEY J A. 1982. *Arch Surg* 78 254-303  
 KEY J A. 1983. *Arch Surg* 79 254-303  
 KEY J A. 1984. *Arch Surg* 80 254-303  
 KEY J A. 1985. *Arch Surg* 81 254-303  
 KEY J A. 1986. *Arch Surg* 82 254-303  
 KEY J A. 1987. *Arch Surg* 83 254-303  
 KEY J A. 1988. *Arch Surg* 84 254-303  
 KEY J A. 1989. *Arch Surg* 85 254-303  
 KEY J A. 1990. *Arch Surg* 86 254-303  
 KEY J A. 1991. *Arch Surg* 87 254-303  
 KEY J A. 1992. *Arch Surg* 88 254-303  
 KEY J A. 1993. *Arch Surg* 89 254-303  
 KEY J A. 1994. *Arch Surg* 90 254-303  
 KEY J A. 1995. *Arch Surg* 91 254-303  
 KEY J A. 1996. *Arch Surg* 92 254-303  
 KEY J A. 1997. *Arch Surg* 93 254-303  
 KEY J A. 1998. *Arch Surg* 94 254-303  
 KEY J A. 1999. *Arch Surg* 95 254-303  
 KEY J A. 2000. *Arch Surg* 96 254-303  
 KEY J A. 2001. *Arch Surg* 97 254-303  
 KEY J A. 2002. *Arch Surg* 98 254-303  
 KEY J A. 2003. *Arch Surg* 99 254-303  
 KEY J A. 2004. *Arch Surg* 100 254-303  
 KEY J A. 2005. *Arch Surg* 101 254-303  
 KEY J A. 2006. *Arch Surg* 102 254-303  
 KEY J A. 2007. *Arch Surg* 103 254-303  
 KEY J A. 2008. *Arch Surg* 104 254-303  
 KEY J A. 2009. *Arch Surg* 105 254-303  
 KEY J A. 2010. *Arch Surg* 106 254-303  
 KEY J A. 2011. *Arch Surg* 107 254-303  
 KEY J A. 2012. *Arch Surg* 108 254-303  
 KEY J A. 2013. *Arch Surg* 109 254-303  
 KEY J A. 2014. *Arch Surg* 110 254-303  
 KEY J A. 2015. *Arch Surg* 111 254-303  
 KEY J A. 2016. *Arch Surg* 112 254-303  
 KEY J A. 2017. *Arch Surg* 113 254-303  
 KEY J A. 2018. *Arch Surg* 114 254-303  
 KEY J A. 2019. *Arch Surg* 115 254-303  
 KEY J A. 2020. *Arch Surg* 116 254-303  
 KEY J A. 2021. *Arch Surg* 117 254-303  
 KEY J A. 2022. *Arch Surg* 118 254-303  
 KEY J A. 2023. *Arch Surg* 119 254-303  
 KEY J A. 2024. *Arch Surg* 120 254-303  
 KEY J A. 2025. *Arch Surg* 121 254-303  
 KEY J A. 2026. *Arch Surg* 122 254-303  
 KEY J A. 2027. *Arch Surg* 123 254-303  
 KEY J A. 2028. *Arch Surg* 124 254-303  
 KEY J A. 2029. *Arch Surg* 125 254-303  
 KEY J A. 2030. *Arch Surg* 126 254-303  
 KEY J A. 2031. *Arch Surg* 127 254-303  
 KEY J A. 2032. *Arch Surg* 128 254-303  
 KEY J A. 2033. *Arch Surg* 129 254-303  
 KEY J A. 2034. *Arch Surg* 130 254-303  
 KEY J A. 2035. *Arch Surg* 131 254-303  
 KEY J A. 2036. *Arch Surg* 132 254-303  
 KEY J A. 2037. *Arch Surg* 133 254-303  
 KEY J A. 2038. *Arch Surg* 134 254-303  
 KEY J A. 2039. *Arch Surg* 135 254-303  
 KEY J A. 2040. *Arch Surg* 136 254-303  
 KEY J A. 2041. *Arch Surg* 137 254-303  
 KEY J A. 2042. *Arch Surg* 138 254-303  
 KEY J A. 2043. *Arch Surg* 139 254-303  
 KEY J A. 2044. *Arch Surg* 140 254-303  
 KEY J A. 2045. *Arch Surg* 141 254-303  
 KEY J A. 2046. *Arch Surg* 142 254-303  
 KEY J A. 2047. *Arch Surg* 143 254-303  
 KEY J A. 2048. *Arch Surg* 144 254-303  
 KEY J A. 2049. *Arch Surg* 145 254-303  
 KEY J A. 2050. *Arch Surg* 146 254-303  
 KEY J A. 2051. *Arch Surg* 147 254-303  
 KEY J A. 2052. *Arch Surg* 148 254-303  
 KEY J A. 2053. *Arch Surg* 149 254-303  
 KEY J A. 2054. *Arch Surg* 150 254-303  
 KEY J A. 2055. *Arch Surg* 151 254-303  
 KEY J A. 2056. *Arch Surg* 152 254-303  
 KEY J A. 2057. *Arch Surg* 153 254-303  
 KEY J A. 2058. *Arch Surg* 154 254-303  
 KEY J A. 2059. *Arch Surg* 155 254-303  
 KEY J A. 2060. *Arch Surg* 156 254-303  
 KEY J A. 2061. *Arch Surg* 157 254-303  
 KEY J A. 2062. *Arch Surg* 158 254-303  
 KEY J A. 2063. *Arch Surg* 159 254-303  
 KEY J A. 2064. *Arch Surg* 160 254-303  
 KEY J A. 2065. *Arch Surg* 161 254-303  
 KEY J A. 2066. *Arch Surg* 162 254-303  
 KEY J A. 2067. *Arch Surg* 163 254-303  
 KEY J A. 2068. *Arch Surg* 164 254-303  
 KEY J A. 2069. *Arch Surg* 165 254-303  
 KEY J A. 2070. *Arch Surg* 166 254-303  
 KEY J A. 2071. *Arch Surg* 167 254-303  
 KEY J A. 2072. *Arch Surg* 168 254-303  
 KEY J A. 2073. *Arch Surg* 169 254-303  
 KEY J A. 2074. *Arch Surg* 170 254-303  
 KEY J A. 2075. *Arch Surg* 171 254-303  
 KEY J A. 2076. *Arch Surg* 172 254-303  
 KEY J A. 2077. *Arch Surg* 173 254-303  
 KEY J A. 2078. *Arch Surg* 174 254-303  
 KEY J A. 2079. *Arch Surg* 175 254-303  
 KEY J A. 2080. *Arch Surg* 176 254-303  
 KEY J A. 2081. *Arch Surg* 177 254-303  
 KEY J A. 2082. *Arch Surg* 178 254-303  
 KEY J A. 2083. *Arch Surg* 179 254-303  
 KEY J A. 2084. *Arch Surg* 180 254-303  
 KEY J A. 2085. *Arch Surg* 181 254-303  
 KEY J A. 2086. *Arch Surg* 182 254-303  
 KEY J A. 2087. *Arch Surg* 183 254-303  
 KEY J A. 2088. *Arch Surg* 184 254-303  
 KEY J A. 2089. *Arch Surg* 185 254-303  
 KEY J A. 2090. *Arch Surg* 186 254-303  
 KEY J A. 2091. *Arch Surg* 187 254-303  
 KEY J A. 2092. *Arch Surg* 188 254-303  
 KEY J A. 2093. *Arch Surg* 189 254-303  
 KEY J A. 2094. *Arch Surg* 190 254-303  
 KEY J A. 2095. *Arch Surg* 191 254-303  
 KEY J A. 2096. *Arch Surg* 192 254-303  
 KEY J A. 2097. *Arch Surg* 193 254-303  
 KEY J A. 2098. *Arch Surg* 194 254-303  
 KEY J A. 2099. *Arch Surg* 195 254-303  
 KEY J A. 2100. *Arch Surg* 196 254-303  
 KEY J A. 2101. *Arch Surg* 197 254-303  
 KEY J A. 2102. *Arch Surg* 198 254-303  
 KEY J A. 2103. *Arch Surg* 199 254-303  
 KEY J A. 2104. *Arch Surg* 200 254-303  
 KEY J A. 2105. *Arch Surg* 201 254-303  
 KEY J A. 2106. *Arch Surg* 202 254-303  
 KEY J A. 2107. *Arch Surg* 203 254-303  
 KEY J A. 2108. *Arch Surg* 204 254-303  
 KEY J A. 2109. *Arch Surg* 205 254-303  
 KEY J A. 2110. *Arch Surg* 206 254-303  
 KEY J A. 2111. *Arch Surg* 207 254-303  
 KEY J A. 2112. *Arch Surg* 208 254-303  
 KEY J A. 2113. *Arch Surg* 209 254-303  
 KEY J A. 2114. *Arch Surg* 210 254-303  
 KEY J A. 2115. *Arch Surg* 211 254-303  
 KEY J A. 2116. *Arch Surg* 212 254-303  
 KEY J A. 2117. *Arch Surg* 213 254-303  
 KEY J A. 2118. *Arch Surg* 214 254-303  
 KEY J A. 2119. *Arch Surg* 215 254-303  
 KEY J A. 2120. *Arch Surg* 216 254-303  
 KEY J A. 2121. *Arch Surg* 217 254-303  
 KEY J A. 2122. *Arch Surg* 218 254-303  
 KEY J A. 2123. *Arch Surg* 219 254-303  
 KEY J A. 2124. *Arch Surg* 220 254-303  
 KEY J A. 2125. *Arch Surg* 221 254-303  
 KEY J A. 2126. *Arch Surg* 222 254-303  
 KEY J A. 2127. *Arch Surg* 223 254-303  
 KEY J A. 2128. *Arch Surg* 224 254-303  
 KEY J A. 2129. *Arch Surg* 225 254-303  
 KEY J A. 2130. *Arch Surg* 226 254-303  
 KEY J A. 2131. *Arch Surg* 227 254-303  
 KEY J A. 2132. *Arch Surg* 228 254-303  
 KEY J A. 2133. *Arch Surg* 229 254-303  
 KEY J A. 2134. *Arch Surg* 230 254-303  
 KEY J A. 2135. *Arch Surg* 231 254-303  
 KEY J A. 2136. *Arch Surg* 232 254-303  
 KEY J A. 2137. *Arch Surg* 233 254-303  
 KEY J A. 2138. *Arch Surg* 234 254-303  
 KEY J A. 2139. *Arch Surg* 235 254-303  
 KEY J A. 2140. *Arch Surg* 236 254-303  
 KEY J A. 2141. *Arch Surg* 237 254-303  
 KEY J A. 2142. *Arch Surg* 238 254-303  
 KEY J A. 2143. *Arch Surg* 239 254-303  
 KEY J A. 2144. *Arch Surg* 240 254-303  
 KEY J A. 2145. *Arch Surg* 241 254-303  
 KEY J A. 2146. *Arch Surg* 242 254-303  
 KEY J A. 2147. *Arch Surg* 243 254-303  
 KEY J A. 2148. *Arch Surg* 244 254-303  
 KEY J A. 2149. *Arch Surg* 245 254-303  
 KEY J A. 2150. *Arch Surg* 246 254-303  
 KEY J A. 2151. *Arch Surg* 247 254-303  
 KEY J A. 2152. *Arch Surg* 248 254-303  
 KEY J A. 2153. *Arch Surg* 249 254-303  
 KEY J A. 2154. *Arch Surg* 250 254-303  
 KEY J A. 2155. *Arch Surg* 251 254-303  
 KEY J A. 2156. *Arch Surg* 252 254-303  
 KEY J A. 2157. *Arch Surg* 253 254-303  
 KEY J A. 2158. *Arch Surg* 254 254-303  
 KEY J A. 2159. *Arch Surg* 255 254-303  
 KEY J A. 2160. *Arch Surg* 256 254-303  
 KEY J A. 2161. *Arch Surg* 257 254-303  
 KEY J A. 2162. *Arch Surg* 258 254-303  
 KEY J A. 2163. *Arch Surg* 259 254-303  
 KEY J A. 2164. *Arch Surg* 260 254-303  
 KEY J A. 2165. *Arch Surg* 261 254-303  
 KEY J A. 2166. *Arch Surg* 262 254-303  
 KEY J A. 2167. *Arch Surg* 263 254-303  
 KEY J A. 2168. *Arch Surg* 264 254-303  
 KEY J A. 2169. *Arch Surg* 265 254-303  
 KEY J A. 2170. *Arch Surg* 266 254-303  
 KEY J A. 2171. *Arch Surg* 267 254-303  
 KEY J A. 2172. *Arch Surg* 268 254-303  
 KEY J A. 2173. *Arch Surg* 269 254-303  
 KEY J A. 2174. *Arch Surg* 270 254-303  
 KEY J A. 2175. *Arch Surg* 271 254-303  
 KEY J A. 2176. *Arch Surg* 272 254-303  
 KEY J A. 2177. *Arch Surg* 273 254-303  
 KEY J A. 2178. *Arch Surg* 274 254-303  
 KEY J A. 2179. *Arch Surg* 275 254-303  
 KEY J A. 2180. *Arch Surg* 276 254-303  
 KEY J A. 2181. *Arch Surg* 277 254-303  
 KEY J A. 2182. *Arch Surg* 278 254-303  
 KEY J A. 2183. *Arch Surg* 279 254-303  
 KEY J A. 2184. *Arch Surg* 280 254-303  
 KEY J A. 2185. *Arch Surg* 281 254-303  
 KEY J A. 2186. *Arch Surg* 282 254-303  
 KEY J A. 2187. *Arch Surg* 283 254-303  
 KEY J A. 2188. *Arch Surg* 284 254-303  
 KEY J A. 2189. *Arch Surg* 285 254-303  
 KEY J A. 2190. *Arch Surg* 286 254-303  
 KEY J A. 2191. *Arch Surg* 287 254-303  
 KEY J A. 2192. *Arch Surg* 288 254-303  
 KEY J A. 2193. *Arch Surg* 289 254-303  
 KEY J A. 2194. *Arch Surg* 290 254-303  
 KEY J A. 2195. *Arch Surg* 291 254-303  
 KEY J A. 2196. *Arch Surg* 292 254-303  
 KEY J A. 2197. *Arch Surg* 293 254-303  
 KEY J A. 2198. *Arch Surg* 294 254-303  
 KEY J A. 2199. *Arch Surg* 295 254-303  
 KEY J A. 2200. *Arch Surg* 296 254-303  
 KEY J A. 2201. *Arch Surg* 297 254-303  
 KEY J A. 2202. *Arch Surg* 298 254-303  
 KEY J A. 2203. *Arch Surg* 299 254-303  
 KEY J A. 2204. *Arch Surg* 300 254-303  
 KEY J A. 2205. *Arch Surg* 301 254-303  
 KEY J A. 2206. *Arch Surg* 302 254-303  
 KEY J A. 2207. *Arch Surg* 303 254-303  
 KEY J A. 2208. *Arch Surg* 304 254-303  
 KEY J A. 2209. *Arch Surg* 305 254-303  
 KEY J A. 2210. *Arch Surg* 306 254-303  
 KEY J A. 2211. *Arch Surg* 307 254-303  
 KEY J A. 2212. *Arch Surg* 308 254-303  
 KEY J A. 2213. *Arch Surg* 309 254-303  
 KEY J A. 2214. *Arch Surg* 310 254-303  
 KEY J A. 2215. *Arch Surg* 311 254-303  
 KEY J A. 2216. *Arch Surg* 312 254-303  
 KEY J A. 2217. *Arch Surg* 313 254-303  
 KEY J A. 2218. *Arch Surg* 314 254-303  
 KEY J A. 2219. *Arch Surg* 315 254-30

- KING, C E, ARNOLD, L, and CHURCH, J G 1922 *Ibid*, 61, 80-92  
 KING, E. S J 1935 *J Path and Bact*, 41, 117-128  
 KIRKMAN, H, and STOWELL, R E 1942 *Anat Rec*, 82, 373-391  
 KISS, F. 1921 *Ztschr f Anat*, 61, 455-521  
 KITE, G. L 1913 *Am J Physiol*, 32, 146-164  
 KLEIN, S 1905-06 *Am J Anat*, 5, 315-330  
 KNISELY, M H 1936 *Anat Rec*, 64, 499-524, 65, 23-50  
 KNOUFF, R A, BROWN, J B, and SCHNEIDER, B M 1941 *Ibid*, 79, 17-38  
 KOCH, J C 1917 *Am J Anat*, 21, 177-298  
 KOLOUCH, F, JR 1939 *Am J Path*, 15, 413-428  
 KORENCHESKY, V 1941 *J Path and Bact*, 52, 341-347  
 KROGH, A 1929 *The Anatomy and Physiology of Capillaries*, New Haven, Yale University Press, 422 pp  
 ——— 1932 *Cowdry's Special Cytology*, 476-503  
 KUGOTA, T. 1929 *Ztschr f Zellforsch*, 9, 457-465

## L

- LAIDLAW, G F 1932 *Anat Rec*, 53, 399-411  
 LANGWORTHY, O R, LEWIS, L G, DEES, J E, and HESSER, F H 1936. *Bull Johns Hopkins Hosp*, 58, 89-108  
 LARSELL O, and DOW, R S 1933 *Am J Anat*, 52, 125-146  
 LATTI, S, and HARVEY, H T 1942 *Anat Rec*, 82, 281-295  
 LAZEREJ B, THOMSON, J D, and HINES, H M 1943 *Am J Physiol*, 138, 357-363  
 LEBLON, C. P 1943 *Anat Rec*, 85, 325 (Abstract)  
 LEJUNED F E, and BAYON, P J 1942 *Laryngoscope*, 52, 891-921.  
 LEWINSKY, W, and STEWART, D 1936 *J Anat*, 70, 349-353  
 LEWIS, D, and LEE, F C 1927 *Bull Johns Hopkins Hosp*, 41, 241-277.  
 LEWIS, THOMAS 1942 *Pain*, New York, The Macmillan Company, 192 pp  
 LEWIS, W H 1922 *Anat Rec*, 23, 177-184  
 LI, P L 1940 *J Path and Bact*, 50, 121-136  
 LIERLE D M, and POTTER, J J 1941 *Ann Otol, Rhinol and Laryngol*, 50, 235-270  
 LITTLE, C C 1943 *Intern Cancer Res Foundation*, Philadelphia, 120 pp  
 LOVBARD, W P. 1911-12 *Am J Physiol*, 29, 335-362  
 LOOMIS, D, and JETT-JACKSON, C E 1942 *Arch Path*, 33, 735-769  
 LOWRY, O H, and HASTINGS, A B 1942 *Cowdry's Problem of Ageing*, 728-755.  
 LUCAS, A M 1930 *Anat Rec*, 45, 230  
 ——— 1932a *Am J Anat*, 50, 141-177  
 ——— 1932b *Cowdry's Special Cytology*, 1, 407-473  
 LUDLUM, S DEW., TAFT, A E, and NUGENT, R L 1931 *J Physiol Chem*, 35, 269-288

## M

- MACCARDLE, R C, ENGMAN, M F, SR and JR 1941 *Arch Dermat*, 44, 429-440; 1943, 47, 335-372  
 MACGREGOR, A 1935-36 *Proc Roy Soc Med*, 29 (2), 1237-1272  
 MACKENZIE, D W, WHIPPLE, A O, and WINTERSTEINER, M P 1941 *Am J Anat*, 68, 397-456  
 MACKLIN, C C 1925 *Ibid*, 35, 303-329  
 ——— 1936 *Arch Path*, 21, 202-216  
 MACKLIN, C C, and M T 1932 *Cowdry's Special Cytology*, 1, 232-333, 1773-1809  
 ——— 1942 *Cowdry's Problems of Ageing*, 185-253  
 MACLEOD, J J R 1924 *Physiol Rev*, 4, 21-68  
 MACNEAL, W J. 1927 *Contributions to Medical Science, dedicated to A S WARTHIN*, Ann Arbor, Mich, Geo Wahr, pp 525-533  
 MAIL, F P 1911 *Am J Anat*, 11, 211-266  
 MALONE, E I 1932 *Cowdry's Special Cytology*, 3, 1403-1420  
 MANN, INA 1932 *Cowdry's Special Cytology*, 3, 1305-1331.  
 MARINE, D 1932 *Cowdry's Special Cytology*, 2, 799-855  
 MARKEFF, J E 1940 *Contrib to Embryol*, Carnegie Inst, of Washington, 28, 219 308  
 MARK, L 1942 *J Exp Zool*, 91, 365-371  
 MARZA, V. B 1931 *Bull d'hist appl*, 8, 85-102  
 MASON, M L, and ALLEN, H S 1941. *Ann Surg*, 113, 424-450

- MASON I 1928 *Am J Path.* 4 181 212  
 — 1930 *Ibid* 6 217 233  
 MAXIMOW A A and BLOOM W 1930 *Textbook of Histology* Philadelphia W B Saunders Company 583 pp (See also later editions)  
 MCCARRELL J D THAYER S and DRINKER C H 1941 *Am J Physiol.* 133 79 81  
 MCCARTHY J F RITTER J S and KLENFNER L 1927 *J Urol* 17 1 16  
 MCCUTCHEON M 1912 *Arch Path.* 34 167 181  
 McLEAN F C and BLOOM W 1941 *Ibid* 32 315 333  
 McMASTER P D 1912 *Bull New York Acad Med* 18 731-77  
 McNALLY W J and STUART E A 1912 *War Med* 2 683 771  
 McPHAIL M H and READ H C 1912 *Anat Rec* 84 51 73  
 MELTZER F I 1931 *Arch. Otolaryngol.* 19 326 333  
 MENEFY C R 1939 *Anat Rec* 75 39-49  
 MEYER J and NECHERLES H 1910 *J A M A* 115 20, 20, 3  
 MEYER J SEIER F and NEUWELT F 1910 *Arch Int Med* 65 171 177  
 MICHELS N A 1933 *Am J Anat* 52 333 393  
 MILLER C O and DENBAR J M 1932 33 *Proc Soc Exp Biol and Med* 30 677 679  
 MILLER I G JR and KUMEROOK R 1932 *Am J Obst and Gynec* 24 19 27  
 MILLER W S 1932 *Histology* 4 173 177  
 — 1932 *Cowdry's Special Cytology* 1 132 150  
 MINOT C R 1922 *J Exp Med* 36 1-7  
 MIXTER C C 1933 *Proc Int Med Chicago*, 9 285-308  
 MOENCH C I 1933 *Am J Obst and Gynec* 25 410-413  
 MOENCH C I and HOLT H 1931 *Am J Obst and Gynec* 22 199 210  
 MOORE C R 1932 *Allen's Sex and Internal Secretions* pp 281 371  
 MOORE C R IRIE D and CALLACHER T F 1930 *Am J Anat* 45 71 107  
 MOORE C R and QUICK W J 1923 *Anat Rec* 26 311  
 MOORE C A 1939 *Washington Univ Med Alumni Quart* 3 121 133  
 MOORE R A 1912 *Cowdry's Problems of Ageing* 495 517  
 MORRIS J R I 1939 *Arch Path* 21 10 26  
 MORTKAM J C CHAMER W and DREW A H 1922 *Brit J Exp Path* 3 179 181  
 MUDD S and H B H 1929 *J Immunol* 17 39 52  
 MULLER O 1922 *Die Kapillaren der Menschlichen Körperoberfläche* Stuttgart J F. G. Fischer  
 MURPHY J B and STUMM I 1919 *J Exp Med* 29 1-15

## N

- NACSONY D 1941 *Arch Mikr Anat u Entw* 100 433-472  
 NEEDHAM J 1941 *Biochemistry and Morphogenesis* Cambridge Univ Press 780 pp  
 NEMOTES I R 1933 *Arch Otolaryngol* 17 38-42  
 NEUMANN C et al 1913 *Proc Soc Exp Biol and Med*, 54 27 28  
 NICHOLSON D 1931 *Laboratory Medicine* Philadelphia Lea & Febiger 433 pp  
 NICOLLE M and MEYER F 1906 *Ann Inst Pasteur* 20 417-418  
 NOEL R 1922 *Thèse à l'université de Paris* Paris Masson et Cie 158 pp  
 NOVIDEZ J F 1941 *Am J Anat* 69 151-189  
 — 1941 *Anat Rec* 82 593 607  
 NOVAK F and FERRIS H S 1928 *Am J Obst and Gynec* 16 499 530  
 NOYES F B 1928 *Dental Histology and Embryology* Philadelphia Lea & Febiger 465 pp

## O

- O'LEARY J J 1929 *Am J Anat* 43 289 316  
 — 1930 *Anat Rec* 45 7 58  
 O'LEARY J J and WALKER N 1934 *Arch Path* 17 291 310  
 OLIVER JEAN 1931 *Proc Int Med Chicago* 10 No 3 11 pp  
 — 1932 *Atlas of the Kidney in Chronic Bright's Disease* New York Paul Hoeber Inc 21 pp  
 OLIVER JEAN and FORT M 1933 *Arch Path* 15 750 771  
 OME F L 1932 *Cowdry's Special Cytology* 1 375-403  
 ORMSBY O S and MANN H 1943 *Diseases of the Skin* Philadelphia Lea & Febiger 1360 pp  
 OTTLEY C M 1941 4 *Brit J Surg* 29 387 391  
 OVERHOLSER M D and NELSON W O 1931 *Anat Rec* 63 (suppl) 31 32

## P

- PAINTER, T S 1924 *Am Nat*, 58, 506-524  
 PAPANICOLAOU, G N 1933. *Am J Anat*, 52, 519-637.  
 PAPPENHEIMER, A M. 1942 *Am J. Path*, 18, 169-181.  
 ——— 1943 *Physiol Rev*, 23, 37-50  
 PARKER, G. H 1932 *Am. J Obst and Gynec*, 23, 619-626  
 PATEK, P. R 1939 *Am J Anat*, 64, 203-249  
 PETERS, J P 1942 *Ann Rev Physiol*, 4, 89-114.  
 PLENK, H. 1932. *Von Mollendorf's Handbuch der mikroskopischen Anatomie des Menschen* 5 (2), 1-234  
 PLUM, C M 1941 *Acta med Scand*, 107, 32-50  
 POLLOCK, W R 1942 *Anat Rec*, 84, 23-27  
 POPA, J. T, and LUCINESCU, E 1932 *J Anat*, 67, 78-107  
 POPPER, H. 1941 *Arch Path*, 31, 766-802  
 POLYAK, S L. 1941 *The Retina*, Univ Chicago Press, 607 pp  
 POYNTER, C W M 1928-29 *Med Clin North America*, 12, 499-505  
 PRICE, P B, SLOAN, H E, JR, and LAROCHELLE, F T 1942 *Bull. Johns Hopkins Hosp*, 70, 26-54  
 PRICKETT, C O, SALMON, W. D, and SCHRADER, G A 1939 *Am. J Path.*, 15, 251-259  
 PROETZ, A W 1932 *Ann. Otol, Rhinol and Laryngol.*, 41, 125-140  
 ——— 1941 *Applied Physiology of the Nose*, St Louis, Annals Publishing Company, 395 pp  
 PRYOR, J W 1936 *Am. J Anat*, 58, 87-101  
 PULLINGER, B D, and FLOREY, W H 1935 *Brit J Exp Path*, 49-61  
 PULLINGER, B D, and PIRIE, A. 1942 *J. Path and Bact*, 54, 341-344

## R

- RAPAPORT, H G, and KLEIN, S 1941. *J Pediat*, 18, 321-327  
 RASMUSSEN, A T 1928 *Endocrinology*, 12, 129-150  
 ——— 1930 *Am J ANAT*, 48, 461-475  
 ——— 1932 *Cowdry's Special Cytology*, 3, 1674-1725  
 ——— 1933 *Am J Path*, 9, 459-471  
 REGEN, E M, and WILKINS, W E 1936 *J. Bone and Joint Surg*, 18, 61-68  
 REISNER, E H 1943 *Arch Int. Med*, 71, 230-256  
 REITAN, H 1941 *Acta Radiol*, 22, 762-779  
 RICHARDS, A G, ANDERSON, T F, and HANCE, R T 1942 *Proc Soc Exp Biol. and Med*, 51, 148-152  
 RICHARDSON, J. R, and HOLMES E M 1942 *Arch Otolaryngol*, 35, 480-501.  
 RICHTER, K M 1942 *J Morphol*, 71, 53-75  
 RIDDLE, O 1935 *Endocrinology*, 19, 1-13  
 RIFGELE, L 1934 *Ztschr f Zellforsch u mikr Anat*, 20, 432-441  
 RIENHOFF, WILLIAM FRANCIS, JR 1940 *Arch Surg*, 41, 487-507  
 RIES, E 1940 *Ztschr. f. mikr-anat Forsch*, 47, 456-466  
 ROBB, J S, and R C 1942 *Am Heart J*, 23, 455-467  
 ROBERTS, J T, WEARN, J T, and BOTEN, I 1941 *Ibid*, 21, 617-633  
 ROBERTSON, H E 1941 *Arch Path*, 31, 112-130  
 ROBINSON, H B G, BOLING, L R, and LISCHER, B E 1942 *Cowdry's Problems of Ageing*, 366-390  
 ROBISON, R 1932 *The Significance of Phosphone Esters in Metabolism*, New York Univ. Press  
 ROSEBERRY, H H, HASTINGS, A B, and MORSE, J K 1931 *J Biol. Chem*, 90, 395-406  
 ROSKIN, GR 1936 *Ztschr f. Zellf u mikr Anat*, 24, 555-613  
 ROSOF, J A 1934 *J Exp Zool*, 68, 121-165  
 ROSS, J F 1943 *New England J. Med.* 228, 454-458  
 ROTHENBERG, R L, and ROSENBLATT, P 1942 *Arch Surg.*, 44, 764-771  
 ROWNTREE, L G, CLARK, J H, STEINBERG, A., and HANSON, A M 1936. *J A M A*, 106, 370-373  
 RUSSELL, W O, and SACHS, E 1943 *Arch Path (in press)*  
 RUSSELL, W O, and GREGORY, W K 1943 *Arch Neurol and Psychiat (in press)*

## S

- SARIN, F R, CUNNINGHAM, R S, ALSTRIAN, C R. and DOAN, C A 1924 *J Exp Med*, 40, 845-871



- THEWLIS, J 1932 Brit Dent J, 53, 655  
 ——— 1936 Brit J Radiol, 9, 300-312  
 THURINGER, J M 1928 Anat Rec, 40, 1-13  
 TOBIN, C E, and WHITEHEAD, R 1942 J Anat, 76, 342-346  
 TOCANTINS, M 1938 Medicine, 17, 175-258  
 TODD, T. W 1932 Cowdry's Special Cytology, 2, 1173-1210  
 ——— 1942. Cowdry's Problems of Ageing, 323-365  
 TOOTHILL, M. C, and YOUNG, W C 1931 Anat Rec, 50, 95-107  
 TOWER, S S 1932 Brain 55 77-90  
 TROTTER, M 1932 Cowdry's Special Cytology, 1, 41-68  
 ——— 1935 Surg, Gynec and Obst, 60, 1092-1095  
 TURNER, C W, and GOMEZ, E T 1933 Univ Missouri Agr. Exp Sta Res Bull., 182, 43 pp

## V

- VAN DER SPRENKEL, H B 1936 J Anat, 70, 233-241  
 VAN DYKE, J H 1941 Anat Rec, 79, 179-209  
 ——— 1944 Anat Rec (in press)  
 VAN GENDEREN, H, and ENGEL, C 1938 Enzymologia, 5, 71-80  
 VAN WEEL, P B 1939 Ztschr f vergl Physiol, 27, 311-315  
 VAN WEEL, P B, and ENGEL, C 1938 Ztschr f vergl Physiol, 26, 67-73  
 VINCENT, S 1925 Quart J Exp Physiol, 15, 313-323  
 VON KOKAS, E 1938-39 Ztschr f vergl Physiol, 26, 74-78  
 VON MOLLENDORF, W 1930 Der Exkretionsapparat, von Mollendorf's Handbuch der mikroskopischen Anatomie des Menschen, 7 (1), 1-307

## W

- WACHSTEIN, M 1932 Ztschr f d ges exp Med, 83, 491-536  
 WAKIM, K G, and MANN, F C 1942 Anat Rec, 82, 233-253  
 WALKER, A M, BOLT, P A, OLIVER, JEAN, and MACDOWELL, M C 1941 Am J Physiol, 134, 580-595  
 WALKER, A M, and OLIVER, JEAN 1941 Am J Physiol, 134, 562-579  
 WALLS, G L, and JUDD H D 1933 Brit J Ophth, 17, 641-675  
 WARTHIN, A S 1924 J Infect Dis, 35, 32-66  
 WAY, S C, and MEMMESHEIMER, A 1940 Arch Dermat and Syph, 41, 1086-1107  
 WEATHERFORD, H L 1929 Am J Anat, 44, 199-281  
 WEATHERFORD, H L, and TRIMBLE, H C 1940 Anat Rec, 77, 487-507  
 WEAVER, H M, and NELSON, W O 1943 Ibid, 85, 51-68  
 WEBB, R L 1931 Am J Anat, 49, 283-334  
 WEBB, R L, and SIMER, P H 1940 Anat Rec, 76, 449-454  
 ——— 1942 Ibid, 33, 437-447  
 WEDDEL, G J 1941 J Anat, 75 (3), 346-367  
 WFED, L H 1923a Am J Anat, 31, 191-221  
 ——— 1923b Ibid, 32, 253-276  
 WFICHERT, C K, and BOYD, R W 1931 Anat Rec, 59, 157-186  
 WFINER, P 1928 Ztschr f mikr-anat Forsch, 13, 197-268  
 WFISKOTTEN, H G 1930 Am J Path, 6, 183-100  
 WELLER, C V 1933 Proc Nat Acad Sci, 19, 318-320  
 WELLS, H GIDEON 1933 Cowdry's Arteriosclerosis, 323-354  
 ——— 1940 J A M A, 114 (2), 2177-2183, 2284-2289  
 WELLS, L J 1936 Anat Rec, 64, 475-497  
 WFNION, C M 1926 Protozoology, New York, Wilham Wood & Co, 2, 1053-1563  
 WHARTON, L R 1932 J Uiol, 28, 639-673  
 WHITHEAD, R 1936 J Anat, 70, 380-385  
 WIFRDA, J L 1912 Am J Anat, 70, 433-453  
 WILLIAMS, G D 1933 Cowdry's Arteriosclerosis, 537-568  
 WILLIAMS, H H, ERICKSON, B N, and MACY, I G 1941 Quart Rev Biol, 16, 80-89  
 WILLIAMS, R G 1939 J Morphol, 65, 17-51.  
 ——— 1941 Anat Rec, 79, 263-270  
 WILLIAMS, R J 1939 Am J Path, 15, 377-384  
 WILLIS, R A 1936 Proc Roy Soc B, 120, 496-498  
 WILSON, E B 1925 The Cell, New York, The Macmillan Company, 1232 pp  
 WINDLE, W I. 1927 J Comp Neurol, 43, 317-356

- WINTERSTEIN M C, THOMAS R M and LECOMPTRE P M 1938 The Biology of the  
*Sciurus*, Springfield Ill. Charles C. Thomas, 142 pp.
- WINTERSTEIN, M M 1942 Clinical Hematology Philadelphia Lea & Febiger 700 pp.
- WISNMAN B K and DOAN C A 1942 Ann Int. Med. 16 107-1117
- WILLOCKI C R 1932 Cowdry's Special Cytology 3 1484-1521
- WILLOCKI G B and DEMPSEY I W 1939 Anat Rec 75 341-363
- WILLOCKI C R. and KING L S 1936 Am J Anat 68 421-472
- WILLOCKI G B and SNYDER, F F 1933 Bull Johns Hopkins Hosp 52 379-387
- WOLBRICH S B 1933 Am J Path 9 (suppl) 689-699
- WOLBRICH S B and HOWE I R 1935 J Exp Med 42 753-777
- 1936 Arch Path and Lab Med 1 1-24
- WOLF J 1940 Ztschr f mikr-anat Forsch 47 361-400
- WOLFE J M, BURBACK F, LANSING W and WRIGHT A W 1942 Am J Anat 70 135-166
- WOLFF D 1934a Ann Otol Rhinol and Laryngol 43 103-217
- 1934b Ibid 43 483-494

## Y

- YOFFEY J M 1941 Lancet 1 529-531
- YOFFEY J M and SULLIVAN I R 1939 J Exp Med 69 133-141
- YOUNG HUGH H and DAVID DAVID M 1926 Young's Practice of Urology Philadelphia  
 W B Saunders Company 1
- YOUNG J L 1942 Physiol Rev 22 318-374
- YOUNG W C 1931 J Exp Biol 8 151-162

## Z

- ZIMMERMAN H W 1923 Ztschr f Anat 68 29-109
- ZOTH O 1923 Irgein d Physiol 22 315-400
- ZWEIFACH B W 1934 Anat Rec 59 83-108
- 1937 Am J Anat 60 473-657
- 1939 Anat Rec 73 475-495
- ZWEMER P I 1936 Ann J Path 12 107-114

# INDEX

References to figures are given in *italics* Where there are several references to text the major one is presented in bold face type

## A

- ABDOMINAL cavity, 154-157  
 Absorption, alcohol, 164  
   by dental enamel, 139  
   fat, 164, 164  
   from alimentary tract, 135, 137  
   from glomerular filtrate, 227  
   from pericardial sac, 79  
   hepatic phases, 197-198, 197, 198  
   through lymphatic endothelium, 84  
   water, 125, 164, 227  
   (*see* Permeability, Phagocytosis, Storage)  
 Abstinence, effect on fertilizing power, 339  
 Achondroplastic dwarfs, 291-292  
 Acidophile cells, blood (*see* Eosinophiles)  
   pituitary, 129, 130, 131  
 Acinous cells of pancreas, 180-185  
   chromidial substance, 181, 182  
   enzymes, 184-185  
   Golgi apparatus, 182-183, 182, 183  
   mitochondria, 180-181, 181  
   neutral red granules, 181, 182, 184  
   secretin influence, 154  
   seen *in vivo*, 184, 184  
   zymogen, 179, 183-184, 183, 184, 185  
 Acne, 385  
 Addison's disease, cortical atrophy in, 124  
 Adenohypophysis, 128  
 Adhesions in peritoneal cavity, 157  
 Adipose tissue, 277 (*see* Fatty tissue)  
 Adjustment, deprivation gradient, 370-371  
   kinds of cellular, 14-15, 14  
   responsiveness of cells, 131, 248, 373, 391  
   spread of labor, 392  
 Adrenalin (*see* Epinephrine)  
 Adrenaltropic hormone, pituitary, 122, 125, 131  
 Adrenals, 121-127  
   adrenotropic hormone, 122, 125, 131  
   as double duty endocrines, 121  
   blood supply, 122, 127  
   chromaffin reaction, 121, 126, 127  
   cortex, 122-125, 121, 122, 123, 124, 190  
   cortical hormones, 125  
   lipoids, 123-125, 123  
   lymphatics, 127  
   medulla, 126-127  
   medullary tumors, 127  
   reticulo-endothelial cells, 125  
   sex hormones, 125  
   vitamin C, 125  
   water content, 127  
   summary, 136  
 Adventitia, arterial, 57, 58-59  
 Aerosol, wetting agent, 374  
 Age, adrenal cortex, 121, 121  
   alimentary tract, 173  
   arteries, 63, 64, 71  
   bone, 292  
   cartilage, 281  
   dermis, 377, 378, 380  
   elastic tissue, 311, 378  
   Age, gingiva, 145, 145  
   hydration, 312  
   influence on thymus, 104-105, 104, 105  
   knee-joint, 302  
   lymph nodes, 106  
   nerve cells, 244-245, 244  
   parathyroids, 121  
   skeletal muscle, 310-312, 311  
   smooth muscle, 305  
   spleen, 106  
   taste buds, 152  
   thyroid, 115, 116  
   water content, 312  
   (*see* Atrophy)  
 Agglomerular nephrons, 230, 230  
 Agranulocytosis, 23  
 Air sacs (*see* Alveoli)  
 Albuminous cells, 149  
 Alimentary tract, lower, 154-176  
   oral cavity, 138  
   salivary glands, 147-149  
   teeth, 139-147  
   tongue, 150-153  
   upper, 137-153  
   summary, 175-176  
 Alveoli, 202, 210-214, 210, 211, 213, 214  
   ducts, 202, 210, 211  
   fluid, 213-214  
   pores, 211, 212, 212  
 Lymphatic bone marrow, 84  
   brain substance, 247  
   cartilage, 280  
   liver lobule, 194  
   permit specialization, 391  
   pulmonary alveoli, 212  
   retina, 259  
   tissues, 84  
 Ambosexual hair, 384  
 Amoeboid movement, 17  
   (*see* Motility)  
 Amitosis, lymphocytes, 30, 31  
 Amniotic cavity, 365  
 Amylase, intestinal, 166  
   pancreatic, 184, 185  
 Androgenic hormone, excretion of, 331  
   influence on skeletal muscle, 310  
   production of, 325, 350  
   zone in adrenal, 125  
 Androsterone, 324  
 Anemia, antipernicious factor, 167  
 Anisotropic bands, 307  
 Anterior cerebral artery, 58  
   chamber of eye, transplants into, 364  
 Antibodies, against erythrocytes, 44, 44  
   exclusion from aqueous humor, 253  
   production in lymph nodes, 93  
 Antipernicious anemia factor, 167  
 Antipressor substance, renal, 229  
 Antrum, cardiacum, 156  
   mastoid, 259-260  
   paranasal sinus, 207  
 Aorta, 57, 58, 58-59



- [illegible]

Blood supply, islets of pancreas, 179-180, 180  
 kidney, 217-218, 218, 219  
 liver, 184, 187-191, 188, 190, 191  
 muscle types compared, 304  
 pars distalis, pituitary, 129  
 respiratory system, 214  
 retina, 257  
 skeletal muscle, 309  
 spleen, 97, 99-101, 100, 101  
 teeth, 142  
 thyroid, 111  
 vasa vasorum, 69  
 (see Avascular tissue)

Blood vessels, 56-75  
 arterioles, 63-66  
 arteriovenous anastomoses, 71, 72  
 capillaries, 66-68, 66, 70, 73  
 correlation of structure and function, 56,  
 71-75  
 elastic arteries, 57-59  
 healing of wounds in, 63  
 heredity, 71  
 moving pictures of, 66  
 muscular arteries, 60-63  
 nervous control, 64, 66, 67  
 regenerative capacity, 56  
 response to infection, 62  
 sinusoids, 73  
 special adaptations, 71-74  
 valves, 70, 71  
 veins, 69-71  
 venules, 68  
 summary, 74-75

Bone, 281-299  
 cancellous, 283, 289, 289, 291  
 compact, 283, 284, 289, 292-296  
 compared with teeth, 300  
 development, 282-292, 282, 283, 287  
 diaphysis, 283, 284  
 endochondral, 283, 284, 285  
 endosteum, 296, 297  
 epiphysis, 283, 284, 287, 291  
 fracture healing, 296-298, 297, 298  
 grafts, 297-298  
 Haversian canals, 294, 295  
 lamellæ, 292, 294, 295  
 lines arrested growth, 286, 286  
 lymphatics, 294  
 mathematical analysis, 287, 288  
 megakaryocytes, 45, 46, 296  
 membrane, 299, 299  
 ossification, 284, 287, 299  
 osteoblasts, 284, 289, 290, 291  
 osteoclasts, 296  
 osteocytes, 295  
 parathormone influence, 292, 292-293  
 periosteum, 284, 296, 297, 298  
 phosphatase, 290  
 polykaryocytes, 53, 296  
 radiostrontium accumulation, 286  
 resorption, 296  
 vital staining of, 285-286  
 vitamin C influence, 292  
 D influence, 286, 292, 293  
 Volkmann's canals, 295

Bone marrow, 49-53, 283, 287, 291  
 differential counts, 53  
 cell lives, 53  
 general properties, 49  
 identification of cells, 50-53  
 neutrophils in, 23  
 numerical increase, 51  
 summary, 55

Bowman's, capsule of nephron, 220, 227

Bowman's, membrane, 252, 253  
 Bright's disease, 229  
 Brilliant cresyl blue staining reticulocytes, 43  
 Broad ligament, 340  
 Bronchi, 202, 209, 215  
 Bronchioles, 202, 209, 209, 210  
 Brunner's glands, 159, 162, 165, 166, 168  
 Bulbourethral gland, 316, 334  
 Bursæ, 300

C

CABOT rings, 42  
 Calcite particles passage through lymphatics, 93  
 Calcium, bone, 281  
 carbonate otoliths, 262-263  
 dentin, 141-142, 142, 143  
 deposits pineal, 134, 135  
 enamel of teeth, 139-140, 139  
 parathormone, 115  
 phosphate precipitation, 290  
 reservoirs, 292  
 rich diet, 292, 293  
 salts solution of, 296  
 Calyx, major, renal, 217  
 minor, 217, 230-231

Canalicular apparatus, pancreatic acinous cells,  
 182, 182  
 Canaliculi of parietal cells, 160, 160  
 Cancer, cells in lymph nodes, 93, 93  
 epidermis, 374  
 mammary glands, 366  
 of prostate, 334

Capillaries, atypical, 72, 73  
 blood, 66-68, 66, 70, 73, 74  
 in cardiac hypertrophy, 78  
 moving pictures, 67  
 permeability, 67, 70  
 Rouget cells, 66, 66  
 skin, 380  
 surface of exchange, 66  
 sympathetic stimulation, 67  
 summary, 75

Carbohydrate diet on liver, 196, 196  
 Carbon, in liver, 192  
 in lymph nodes, 91-93, 91, 92  
 macrophages, 36, 36, 92  
 monocytes, 36, 36  
 phagocytosis of, 36, 36  
 Carbon dioxide, effect on motor end plates, 306  
 Carboxylpolypeptidase, 184  
 Carcinogens, 366  
 Carcinoma, stroma of, 270  
 Cardiac glands, 158, 158  
 Cardiac muscle, 312-315  
 blood supply, 77-78, 78  
 bundles, 76, 77  
 compared with other types, 304  
 hypertrophy, 78, 78  
 intercalated discs, 313, 313  
 lipofuscin, 315  
 lymphatics, 78, 79  
 protection of, 81-82  
 segmentation, 314  
 summary, 315

Cardiovascular system, broadcasting, 391  
 Carotid arteries, 59  
 Cartilage, 280-281  
 articular, 283, 300  
 chondromucoid of, 280  
 dormant, 291  
 elastic, 281  
 epiphyseal plate, 283, 285, 292  
 fibrocartilage, 281



- regeneration, vascular endothelium, 69  
   Necrosis, Involution)  
 rehydration, of bile, 199  
 androsterone, 324  
 drotheelin, 324  
 nervation of skeletal muscle, 310  
 er's cells, 263  
 olune cells, salivary, 149  
 iravone, 234  
 lrites, contrasted with axones, 234  
 ervation, effect on testicle, 325  
 im, 138, 140-141  
 mpared with bone, 300  
 percalcification, 140, 141  
 pocalcification, 140, 141  
 atrix, 141  
 rathyroid hormone, 141, 143  
 dium fluoride, 141, 142  
 bules, 140-141, 140, 141  
 tamin D, 141  
 tistry, maturity of, 146  
 natology, 367  
 docrines in, 376  
 nis, 377-383  
   e differences, 377, 378, 380  
   ood vessels, 380, 380, 383  
   romatophores, 379  
   astic fibers, 378  
   urs, 383-385  
   rause's end bulb, 379  
   mphatics, 381, 383  
   eissner's corpuscle (tactile), 372, 379, 380  
   rve fibers, 380  
   un sensation, 379, 380  
   urs papillaris, 370, 388  
   rcticularis, 370, 378, 388  
   emet's membrane, 252, 253  
   oxification, by eosinophiles, 29  
   terium marked fatty acids, 278  
   elopment, bone, 282-292, 282, 283, 287  
   ir, 259-261  
   e, 254  
   dney, 219  
   betes, discovery of, 187  
   etogenic hormone, pituitary, 131  
   physis, 283-284, 283  
   rthrosis, 299  
   , cholesterol, 124  
   gh sugar (liver), 196  
   crential leucocyte counts, 21-22  
   rneth, 24, 24  
   one marrow, 55  
   fluence of emotion on, 22  
   ormal variations in, 22  
   hilling, 27  
   erentiating intermitotics, 54, 244, 321, 371  
   estive tract (*see* Alimentary)  
   eptidase, 162, 166, 167, 184, 185  
   ance receptors, 248  
   ributing arteries, 60  
   rticulosis, 173  
   oxidase, 373, 374-375  
   gs, influence of, atropine, 184, 184  
   lurpine, 28, 167, 183, 184, 208  
   Hormones)  
   list (concept of blood, 38  
   ts bile, 188, 189, 190, 198, 199  
   olymphatic, 259, 260, 261  
   cretory, 150  
   tercalated, 148, 149, 149  
   nergetic, 149, 178, 179, 179  
   livary glands, 119  
   retory, 148, 149, 149  
   d glands, 367, 368, 370
- Ductus cochlearis, 259, 260, 263  
   endolymphaticus, 259, 260, 261  
 Ductus (canalis) reuniens, 260, 261  
 Duodenojejunal flexure, 167  
 Duodenum, 165, 165, 170  
 Dwarfs, 291-292  
 Dynamic polarization, nervous 242-245
- ## E
- EAR, 259-264  
   audition, 263  
   cochlea, 260, 262-263, 262  
   cochlear fenestra 259, 260, 262  
   Deiters cells, 263  
   development, 259-261  
   ductus, cochlearis, 259, 260, 263  
     endolymphaticus 259, 260, 261  
     reuniens, 260, 261  
   endolymph, 261  
   endolymphatic duct, 259, 260, 261  
   equilibration, 262-263  
   Eustachian tube, 259, 260  
   hair cells, 262, 263  
   labyrinth, 260  
   macula, 262  
   mastoid antrum, 259-260  
   membrana basilaris, 262, 263, 263  
     tectoria, 262, 263, 263  
   organ of Corti, 263, 263  
   ossicles, 259, 261  
   perilymph, 261, 262  
   receptors, 262-263, 263  
   sacculus, 259, 260, 261, 262  
   saccus endolymphaticus 259, 261  
   scala tympani, 262, 262  
     vestibuli, 262, 262  
   semicircular canals, 259, 260, 261, 262  
   sound transmission 262  
   utricle, 259, 260, 261, 262  
   summary, 263-264  
 Eclampsia, necrosis in, 195, 195  
 Economy and stability, 392-393  
 Eczema, 373  
 Edema, 276  
   with age, 312  
 Egg white injections, lymphocytic response 32  
 Elastic arteries, 56, 57, 58, 57-59, 74  
   ageing, 63  
   calcium in 60  
 Elastic cartilage, 281  
 Elastic fibers, 57-58  
   ageing, 63, 64  
   aorta, 57, 58  
   compared with collagenic, 271, 272  
   durability 394  
   in different blood vessels, 56  
 Electron microscopie, collagenic fibers, 267, 268  
 Emotion, influence on, lymphocyte counts, 22  
   salivary secretion, 150  
 Enamel, compared with bone, 300  
   dental 139, 139, 139-140  
 Endarteries kidney, 218  
   Purkinje system, 81  
   retina, 259  
 Endocardium, 77  
 Endocrine system 109-136  
   adrenals 121, 127  
   double duty 121  
   duodenum 167  
   kidney 229  
   non-specificity, 136  
   ovary 318, 319



Eye, ora serrata, 250, 251  
 orbiculus ciliaris, 251  
 riboflavin deficiency, 252  
 retina, 250, 251, 254, 255-259, 256, 258  
 rhodopsin, 257  
 sclera, 250, 251  
 theories of vision, 257  
 tissue transparency, 254  
 vitreous body, 250, 254  
 zonula ciliaris, 250, 254

## F

FACTORS of safety, adrenal cortex, 394  
 capillary surplus, 66  
 enzyme duplication, 176  
 kidneys, 394  
 liver, 394  
 lungs, 394  
 nervous, 243  
 pancreas, 394  
 renal, 229  
 thyroid, 394  
 Fallopian tubes, 350-352, 351, 365  
 cycle, 363, 364  
 Fasting, digestive tract, 170  
 influence on Paneth cells, 166  
 thyroid and parathyroid, 120  
 Fat, absorbed by lacteals, 84  
 adrenal cortical, 124  
 anterior pituitary hormone, 280  
 as chylomicrons, 47-48, 47  
 in hepatic lobules, 196  
 in intestinal epithelial cells, 164, 164  
 lipocaine hormone, 187, 280  
 storage of, 278, 280  
 Fat cells, 105, 277-280, 277  
 cyclic changes in, 278, 278  
 identification, 86, 86  
 influenced by blood stream, 279-280, 279  
 Fatty acids, marked by deuterium, 278  
 Fatty tissue, 277-280  
 obesity hereditary, 280  
 regional distribution, 278, 280  
 Feeding (see Diet)  
 Female reproductive system, 340-366  
 external genitalia, 360  
 Fallopian tubes, 350-352, 351, 363  
 hormone production, 348-350  
 integration of activities, 363-364  
 mammary glands, 358, 359, 360, 361, 361-362  
 nervous versus hormone regulation, 364  
 ovarian architecture, 340-341, 341  
 oögenesis, 341, 341-348, 344, 346, 347  
 placenta, 365, 365  
 testis and ovary compared, 349-350  
 uterus, 353-359, 353, 355, 356, 363  
 vagina, 377, 359-360, 363  
 summary, 365-366  
 Femoral artery, 58  
 Ven. vasa vasorum, 69  
 Femur, development of, 282-285, 282, 283  
 Ferments (see Enzymes)  
 Fertilization, location of, 351  
 Fetal cortex, adrenal, 121  
 Fibrin, as source of collagenic fibers, 268-269  
 Fibrinogen, 200  
 Fibroblasts, 266, 270, 273, 274, 275, 276  
 compared with epithelial cells, 276-277  
 food substances for, 27  
 growth rate, 271, 275  
 identification, 273

Fibroblasts, moving pictures, 277  
 relation to other cells, 58  
 Fibrocartilage, 281  
 Fibroglial fibers, 268, 269, 270  
 Fibrosis, as age change, 270  
 definition of, 276  
 Filiform papillae, 151  
 Filtration, renal, 227  
 Final common path, 243, 248  
 Fixation shrinkage, artery, 60, 61  
 dermis, 377, 378, 379-380  
 Graafian follicle, 346  
 large intestine, 171  
 muscle nuclei, 304  
 small intestine, 163  
 spleen, 95  
 Fluids, alveolar, 211, 213  
 aqueous humor, 253  
 bile, 199  
 blood, 12, 12-13  
 "bucket brigade," 349  
 cerebrospinal, 246-247  
 endolymph, 261-262  
 for ciliary action, 204, 205  
 glomerular filtrate, 227  
 interstitial, 15, 16  
 intracellular, 15  
 liquor folliculi, 344, 345-346  
 lymph, 11, 12, 16  
 perilymph, 261-262  
 peritoneal, 155  
 principal ones, 12-16  
 seminal, 337  
 subarachnoid, 247  
 synovial, 302  
 tissue, 13, 13-16, 25-26, 253  
 ventricular, 246  
 Fluorescence, hairs, 382, 383  
 hepatic vitamin A, 191, 192  
 islets of pancreas, 179  
 lachrymal gland, 252  
 ovary, 348  
 skin, 367  
 sweat glands, 386, 386  
 thyroid colloid, 110  
 Follicle stimulating hormone, 349, 350  
 Follicles, Graafian, 341, 342-345, 344, 346, 348  
 Follicular, atresia, 345-346  
 hormone, 348  
 Food products, circulation of, 135, 137  
 Foreign body giant cells, 296  
 Fractures of bones, 296-298, 297, 298  
 Freezing, 393  
 Friction reduction by, peritoneal surface, 176  
 pleural surface, 216  
 serous fluids, 154, 204  
 synovial fluid, 302  
 Frontal sinus, 207  
 Fundic glands (see Gastric)  
 Fungiform papillae, 151  
 Fuscine, retinal pigment, 255, 257

## G

GALL BLADDER, 198, 199  
 Ganoblasts, 110  
 Gastric glands, 157, 158, 159-161, 159, 160, 161  
 Germinal zone, adrenal cortex, 121  
 Giant cells, foreign body, 296  
 megakaryocytes, 45, 46, 50, 206  
 polycaryocytes, 53, 206  
 Giants, pituitary, 292  
 Gingiva of teeth, 138, 145, 145

- H**
- Haversian canal 79 80  
Hazardous cancer metastasis 393  
dominance of brain 393  
excesses 393  
limphatics in virus extension 60  
permeability of appendix 172 393  
respiratory system 201  
sex hormones and carcinogens 94 393  
urine traversing prostate 59  
**Heart** 76 82  
conductive system of 313  
endocardium 77  
epicardium 77  
lymphatics 79  
maintenance 51  
muscle bundles 41  
myocardium 77 78  
pericardium 77  
Purkinje system 80  
sino-ventricular system 80 81  
valves 79  
in primary 82  
**Heat** (*see* temperature)  
**Heavy hydrogen** 27  
**Hematopoiesis** 49  
**Hematologists** meets of 38  
**Hemoconia** blood dust 48  
**Hemoglobin** localization 4)  
muscular 308  
recognition 17  
**Hemolymp nodes** 83  
**Hemorrhagic purpura** spleen in 27  
**Hemosiderin** 101 10  
**Heparin** 29 274  
and basophil leucocytes 29  
**Hepatotoxin muris phagocytized by macrocytes** 52  
**Heredity** baldness 38,  
blood pressure 71  
chromosomes X and Y 37  
extrachromosomal factors 350  
hair color 354  
hypertension 71  
nuclear membranes 311  
obesity 280  
**Hermaphrodites** 318  
**Hibernation** influence on brain fluid 2  
**Hila renal** 27  
Hilum bundle of 50 80  
**Histamine** effect on gastric gland 161 171  
**Histiocytes** 274  
**Histopecterography** 373  
**Histiocytes** 340  
**Homeostatic stabilization** 15  
**Hormones** adrenotropic (adrenals) 17  
125 131  
alimentary tract 13  
androgens 310 324 325 331 340  
anrostosterone 374  
antidiuretic 226  
cholesterol kinase 167  
cortisol 324 340 372  
cortical 125 226  
dihydroxyandrosterone 371  
diabetogenic 131  
dihydrotestosterone 321  
of pregnancy 121 127  
estradiol benzoate 376 376  
estrogen 348 349  
estrogens 30 324 340 377  
folic stimulating 317 340  
follicular 315  
gonadal tissue 171 350 350  
growth 131

- Hormones, inhibition by, 350  
 insulin, 185-187  
 lactogenic, 131, 362  
 lipocarc, 187, 280  
 luteinizing, 349, 350  
 luteosterone, 324  
 neurohormones, 277  
 parathormone, 115, 120  
 parathyrotropic, 131  
 pituitary, 131, 135  
 pituitrin, 135  
 progesterin, 324  
 progesterone, 125, 324, 348, 365  
 renin, a pressor substance, 229  
 secretin, 167  
 sex, 125  
   hormone terminology, 324  
 steroid, 125  
 testis, 329  
   hormone indicator, 333  
 testosterone, 125, 324, 348, 374, 376, 376  
 theelin, 330, 330, 324, 360, 362  
 theelol, 324  
 thymoxidin, 107  
 thyrotropic, 109, 114, 114  
 thyroxin, 109, 280  
   zoological non-specificity, 136  
 Howell-Jolly bodies, 42, 43  
 Hyaline bodies of Herring, 133, 133  
   cartilage, 281  
 Hydrochloric acid, gastric, 160  
 Hydrogen, heavy, 278  
 Hydriokollag injection of lymphatics, 83  
 Hydropic degeneration, pancreatic islets, 186, 187  
 Hypercalcification, dentin, 140, 141, 142, 143  
 Hyperinsulinism, 185  
 Hyperparathyroidism, 119  
 Hyperplasia, alveolar, 312, 314 (*see* Tumors)  
 Hypertension, paroxysmal, 127  
 Hypertrophy, adrenal cortex, 122, 124  
   cardiac, 77-78  
   gouters, 110-112, 113  
   nucular, 197  
   obesity, 280  
   ovarian arteries, 343  
   ovaries, 349  
   parathyroid, 116, 119, 120  
   prostate, 334  
   renal units, 229, 230  
   seminal vesicles, 330, 330  
   smooth muscle, 305, 305  
   (*see* Tumors)  
 Hypertonic solution, 42  
 Hypocalcification, dentin, 140, 141, 142, 143  
 Hypothyroidism, 109, 112, 113  
 Hypophysectomy, effect on thyroid colloid, 110  
 Hypophysis (*see* Pituitary)  
 Hyperthyroidism, 109, 112, 113  
 Hypotonic solution, 42
- I**
- Ilium, 167, 168, 168, 169, 170  
 Immunization, against erythrocytes, 41, 41  
   Lymph nodes in, 93  
 Implantation, of ovum, 353, 354  
   anterior lobe pituitary, 349  
   traumatic of epidermis, 372  
   (*see* Transplantation)  
 Impulse, nervous, 233  
 Insatiation (*see* Fasting)  
 Incisures of Schmidt-Lantermann, 237  
 Incus, 259  
 India ink, phagocytosis of, 36  
 Infection, responses of blood vessels, 62  
 Infectious mononucleosis, 33  
 Infiltration, lymphocytes, digestive tract, 170  
   neutrophils, 26  
 Inflammation, healing fracture, 297  
   synovial membrane, 301, 301  
   tracheal epithelium, 208, 208  
   vascular changes, 61, 62  
 Infundibulum, 133, 133  
 Innervation (*see* Nerve supply)  
 Innominate artery, 58  
 Insulin production, 185  
 Integration, by hemoglobin transport, 45  
   connective system, 265  
   endocrine broadcasting, 109, 136  
   nervous, 233  
   *via* blood stream, 137, 137  
   with environment, 388  
 Intercalated discs, 313, 314  
   ducts, salivary, 148, 149, 149  
 Intermittent cells, bone marrow, 54  
   myelocytes, 23  
 Internal auditory meatus, 259  
 Interstitial cells, 324-325  
   ovary, 347  
   testis, 318, 320, 321, 322  
 Interstitial fluid, 15, 16  
 Intervertebral discs, 281  
 Intestinal glands, 162  
 Intestine, contracted and distended, 162, 163  
   dissection of, 162  
   duodenum, 165, 165, 170  
   ileum, 167-168, 168, 169, 170  
   jejunum, 167  
   movements of villi, 162  
   small, 162-170  
   surface epithelium, 163  
 Intima, arterial, 57, 58, 59  
 Intrinsic antipernicious anemia factor, 167  
 Inulin, renal clearance, 227  
 Invertase, 175  
 Involution, thymus, 105, 105  
   (*see* Degeneration, Necrosis)  
 Iodine, influence on thyroid, 109, 111  
   radioactive, 112  
 Iris, 250, 254  
 Iron metabolism, 103  
 Islets of Langerhans, 177-178, 177, 178  
   blood supply, 179, 180  
   cell types, 185, 187, 186  
   fluorescence, 179  
   insulin production, 185-187  
   *seen in vivo*, 185  
   supravital staining, 177, 177  
 Isotonic solution, 42  
 Isotropic bands, 307
- J**
- Janus green staining, fibroblasts, 273, 275  
   mitochondria, 20, 180, 181  
   of acinous cells, 181  
   of leucocytes, 57  
 Jejunum, 167, 167  
 Juxta medullary zone, adrenal cortex, 121  
 Juxtaglomerular apparatus, 220, 223, 229
- K**
- Kidney, 217, 230  
   artery, 217, 218, 219





- Lymphocytes, amitosis, 30, 31  
 antibody production, 93  
 appearance unstained, 19, 19  
 azurophilic granules, 30  
 emotion, influence on, 22  
 fasting, influence on, 170  
 heat, influence on, 31  
 motility, 35  
 neutrophils resembling, 26  
 parasites in, 30, 36  
 phagocytosis by, 31, 33  
 sizes of, 30  
 small, 52, 53  
 transforming into macrophages, 32, 32  
   into monocytes, 32-34  
 Wright's stain, 21, 41  
 Lysosome of lachrymal fluid, 252

## M

- Maceration, kidney, 219  
 muscle, 303  
 thyroid, 109  
 Macrocytes, 41  
 Macrophages, 36, 274  
   compared with fibroblasts, 274  
   containing carbon, 36, 91-92  
   digesting erythrocytes, 44, 44  
   from lymphocytes, 32  
   from monocytes, 36  
   in lymph nodes, 91, 92  
   peritoneal, 155  
   relation to non-granular leucocytes, 38  
   stained with janus green, 37  
   subcutaneous, 274  
 Macula densa, renal, 220, 223, 229  
   vestibular, 262  
 Magnesium deficiency, cutaneous, 373  
 Malaria parasites, 41  
 Male reproductive system, 316-339  
   bulbourethral glands, 316, 334  
   ductuli efferentes, 322, 326  
   epididymis, 327  
   hormone production, 324-326  
   penis, 316, 334-336, 337  
   principal and accessory parts, 316, 316  
   prostate, 316, 331, 332, 333, 331-334  
   rete testis, 317, 317, 318, 326  
   semen, 336-338, 336  
   sex hormone terminology, 324  
   spermatogenesis, 319-324  
   testis architecture, 316-317  
   tubuli recti, 318, 326  
   vas deferens, 316, 327  
   vesiculae seminales, 316, 328, 329 331, 329, 330  
   summary, 338-339  
   (see Sperms)  
 Mallus, 259  
 Malpighian bodies of spleen, 95 96, 97  
   variation in, 98, 98  
   corpuscles, renal, 224  
 Maltase, pancreatic enzyme, 184  
 Mammary glands, 361-363  
   after parturition, 359  
   castrate atrophy, 360  
   compared with prostate, 362  
   with thyroid, 361  
   cycle, 361, 361  
   inactive, 358, 361  
   involved 5 days, 379  
   lactating, 358, 361  
   luteogenic hormone, 362  
 Mammary glands, pro gravid change, 362  
   theelin and corporin, 360, 362  
   theelin (estrone) effect, 360, 362  
   tumors, 362  
 Margin of safety, capillary surplus, 66  
   enzyme duplication, 176  
   nervous, 243  
   renal, 229  
 Mast cells (see Basophile leucocytes) connective tissue, 273  
 Mastoid antrum, 259-260  
 Maxillary sinuses, 207, 260  
 Meckel's diverticulum, 168-169  
 Media, arterial, 57, 59  
   calcium in, 60  
 Megakaryocytes, 45, 46, 50, 296  
 Megaloblasts, 41, 46  
 Meibomian glands, 385  
 Meissner's tactile corpuscle, 372, 379, 380  
 Melanin, epidermal, 372, 373, 374  
   hair, 384  
   phagocytes of, 379  
   retinal, 255  
 Melanoblasts, 374  
 Melanomas, 375  
 Membrana, basilaris, 262, 263, 263  
   tectoria, 262, 263, 263  
 Membrane bones, 299, 299  
 Membranes, arachnoid, 245, 245, 246  
   basilaris, 262, 263, 263  
   brain, 245-246  
   leptomeninges, 245-246, 246  
   pachymeninx, 245  
   pancreatic acinous cells, 183, 184  
   pia mater, 245, 245, 246  
   plasma, 243  
   tectorial, 262, 263, 263  
   thyroid cells, 114, 114  
 Memory, basis of, 248  
 Menstrual cycle, 353-358, 363  
   activation of sweat glands, 386  
   in transplants, 364  
   ischemia, 357  
   leucocytes, 354  
   menstrual period, 354-358, 376, 363  
   nerve section, 364  
   postmenstrual period, 358  
   pro gravid period, 354, 355, 363  
   proliferative period, 353-354, 355, 363  
 Menstruation, leucocytosis, 26, 26  
 Merkel cells, 375  
 Mesenchyme, 260, 265  
   in bone development, 282, 283, 299  
 Mesenteric lymphatics, moving picture, 175  
 Mesenteriole of appendix, 171  
 Mesothelium, germinal, 341, 341  
   peritoneal, 156 157  
   scrotal, 317  
 Metabolism, basal, 112  
   carbohydrate, 196  
   iron, 103  
   oxidative, 196  
   water, 232  
   (see Fat)  
 Metaplastic ossification, 299  
 Metastasis in lymph nodes, 93, 93  
 Microcytes, 41  
 Microdissection, renal, lobule, 221  
   muscle, 308  
   nerve fibers, 237  
 Microfilariae, passage through lymphatics, 93  
 Microglia, 245  
 Micromercuration, elastic artery, 60  
   nerve cells, 240, 242

Ova (eggs) + blastula + 3, 3 354

  Fecundation 317

  Fertilization 352

Ovarian follicles 300

Ovary 310 340 341

  arteries of 345

  corpus albicans 341 342 343

  lutea 341 342

  corpus striatum 343

  interovulatory 341

  verum 341

  cervix 341 342 343

  cycle 341 342

  endometrium 345

  follicles 345

  follicular atresia 341 342

  germinal mass 341 342

  granulosa follicles 341 342 343 344 345 346 347 348

  graft 340

  influence of temperature 340

  interfollicular tissue 347

  interstitial cell 347

  ova 341 342 343 344

  ovogenesis 341 342 343 344 345 346 347

  pituitary implant 341 342

  primary oocytes 347

  primordial follicles (germinal cells) 341 342 343 344 345

  temp. mature cell of 340

  testosterone 345

  theca externa 341 342

  zona granulosa 341 342

  zona pellucida 341 342

Oogenesis 341 342 343 344 345 346 347

Ovulate 341 342 343 344

## P

PARATHYROID 24

Parasitization 200 201

  intestine 171

  liver 200

  tooth 171

Parasites 171 187

  a in tissue 180 181 182 183 184 185 186 187

  at the infection 181

  the body 179 180

  parasites 187

  the body 179 180

  the body and excretory parts 177

  the body 177

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

  the body 177 178 179 180 181 182 183 184

Parathyroid (oxyphilic cell) 11 115 116

  principal cells 11 115

  temperature influence 115 116

  tumors 119

Parathyroid hormone pituitary 131

Parathyroid cell 131 160 161

  eye 131

Parathyroid gland 147 148

Parathyroid pituitary 125 131 132 133 134 135

  intermedia 125 131 132 133

  nervous 131 132 133 134 135

  tubercles 125 131

  thyroid 125 131

Parathyroid 316 331 332 333

  Pepsin 160 161

  cell 160 161

  Pepsinogen 161 162

  Pepsinase enzyme of pyloric gland 161

Performance and replacement 341

Pericardium 20

Perichondrium 200 201

Pericytes 66 67

Perilymph 241 242

Perimurium 276

Perimurium space 20 21

Pericardial membrane (tooth) 111 112

Pericardium 284 285 286 287 288

Pericardium 111 112

  exudate cell 111

  omentum 111

  regional differences 111

  surface 111

Perivascular channel 20 21

Permeability capillaries 60 61 62 63

  lipids and 63

  of different blood vessel 63

  vascular endothelium 111 112

  venules 65 66

  vitamin P 17 18

Peroxidase reaction 20

Perforated patches 88 165

Phagocytosis by eosinophiles 20 21

  by Kupfer cells 19

  by lymphocytes 31 32

  by monocytes 31 32 33

  part in exudate cell 11 12

  epithelial 214

  function of 35

  induced 379

  melanin 379

  of carbon 37 199

  of erythrocytes 41 42

  of spermatozoa 26 27

  of streptococci 31

Pharynx 153 207

Phosphatase in bone 200

  intestinal epithelial cell 161

Phosphorus and nickel 190

Physiological age 207

Physiological 211

  matter 211

Physiological epithelial 211 212

  factors 211

Physiological 116 163

  cornea 211

  lumen 211 212

  hemoglobin 17 45 101

  hemoglobin 101 102

  lens 211

  lymphocytes 329

  lymphocytes 316

  muscle 211

  muscle 211

  muscle 211

  muscle 211

  muscle 211

- Pigments, rhodop-in, 257  
   visual purple, 255, 257  
 Pilocarpine, effect on, enterochromaffin cells, 167  
 eosinophiles, 28  
   pancreatic acinous cells, 183, 184  
   islet cells, 184  
   tracheal epithelium, 208  
 Pinea, 134, 135  
 Pinelectomy, 135  
 Pinocytosis, drinking by cells, 184  
 Pituitary, 127-135  
   acidophile cells, 129, 130, 131, 133  
   anterior lobe (distalis), 128-133, 128, 129, 130, 131  
   basophile cells, 129, 130, 131, 133  
   chromophobe cells, 129, 130, 130, 133  
   divisions of, 128, 128, 131  
   hormones, 131, 135  
   hyaline bodies, 133, 133  
   implants, 349, 349  
   pars intermedia, 128, 133  
     nervosa, 128, 132, 133, 133, 135  
   posterior lobe, 128, 133  
 Pituitum, origin of, 135  
   vasoconstrictor, 83  
 Placenta, 365, 365  
 Plasma cell, 274, 276  
   in lymph node, 92  
   in tongue, 151  
   relation to other cells, 38  
 Plasma membrane, molecular composition, 243  
 Platelets, 41, 45-47, 46  
   deficiency, spleen in, 96  
 Pleural cavity, 216  
 Plexus, myentericus, 157, 165, 174  
   submucosus, 157, 174  
 Plicae circulares, small intestine, 163, 163  
 Poikilocytes, 41  
 Polar body, 347  
 Polarized light and, cortical lipoid, 122, 125  
   nerve fibers, 237, 238  
   striated muscle, 307  
 Polarization, dynamic, 242-245  
 Poliomyelitis, skeletal muscular changes, 310  
   virus, 205  
 Polychaetes, 53, 296  
 Porphyrin, excretion of, 252  
 Portal canals, of liver, 85, 188, 188, 193, 194, 195  
 Postmitotic cells, nerve cells, 244  
   neutrophiles, 23  
   normoblasts, 42  
 Postmortem changes, renal, 221  
 Potassium sulphocyanide, 60  
 Pudentia, 140, 141  
 Pregnane, hypertrophy of uterine muscle, 305, 307  
 Pressor substance, renal, 229  
 Primitive blood cells, 52  
 Proenzymes, 185  
 Progesterone, 125, 324, 318, 365  
 Progestin, 321  
 Prostate, 316, 331 331-331, 332, 333  
   as testis hormone indicator, 333, 334  
   compared with mammary glands, 332  
   with thyroid, 332  
 Protection by, antibody production, 93  
   clarity clearance, 201-205, 204, 205, 352  
   digestive secretions, 175  
   glottis closure, 207  
   friction reduction, 154, 176, 204, 216, 302  
   hormonal and nervous control blood vessels, 304  
   labor regulation, 302  
   mucus, 175-206  
   phagocytosis, 34-35, 38  
   replacement, 393  
   safety mechanisms, 394  
   skin, 387  
   sneezing reflex, 207  
   sphincters, 175  
   stabilizing mechanisms, 392-393  
   (see Regeneration, Regulation)  
 Protein, in plasma membrane, 243  
   iron containing, 239  
 Protozoan and tissue cells compared, 14, 137  
   parasites in monocytes, 35, 36  
 Prussian blue in endolymphatic duct, 261  
 Puberty, influence on, lymph nodes, 106  
   spleen, 106  
   thymus, 106  
 Purkinje cell of cerebellum, 235, 244, 245  
   muscle, 80, 314, 315  
   compared with other types, 304  
 Pyloric antrum, 156  
   glands, 162  
   valve, 156  
 Pylorus, 156  
 Pyramid, renal, 217
- ## R
- RADIAL artery, 58, 71  
 Radiation, effect on mast cells, 274  
 Radioactive, iron tagging of erythrocytes, 43  
   iodine in thyroid, 112  
   phosphorus in enamel, 139  
   sodium in placental transfer, 365  
   strontium in bone, 286  
 Radio autographs, 112, 286  
 Radium, effect on testis, 325  
 Ranvier, nodes of, 237, 237, 238  
 Rathke's pouch, 133  
 Reactions chromaffin for epinephrine, 121, 126 127  
 Receptors, cochlear, 262, 263  
   distance, 218  
   skin, 233, 233  
   vestibular, 262  
   visual, 256, 257, 258  
   (see Sense organs)  
 Rectum, 173  
 Red blood cells, 40-45  
   basophilic stippling, 41  
   Cabot rings, 42  
   erythroblasts (megaloblasts), 41, 51  
   hyperchromic macrocyte, 41  
   life history, 42-44  
   malarial parasites in, 41  
   microcyte, 41  
   normoblasts, 41, 42, 43, 50, 51, 52  
   phagocytosis of, 41, 44, 100  
   poikilocytes, 41  
   reticulocytes, 41, 42-43  
   service rendered, 45  
   summary, 48  
 Reflex, simple arc, 233  
   sneezing, 207  
 Regeneration (healing), blood vessels, 56, 63  
   collagenic fibers, 268-270, 269  
   corneal epithelium, 253  
   epidermis, 371  
   fractures of bone, 296-298, 297, 298  
   liver lobules, 197  
   mesothelium (replacement), 156  
   nasal mucous membrane, 205-206, 206  
   nerves, 310  
   spermatogenesis, 322-324

Large craters (beating) in school 152  
(see replacement)

Lactate 141 142 also mean 141 137

endoneurial region 391

lateral rotation 397

term 233

reflexes 311 312 313

reflex from 313

replacement 391

stage 313

temperature 49 322 324 340

gastrovascular structures appendix 1 172

er lymphatic duct 1 172

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

epi 201

Lactate lines of 132 140

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

lactate 132

- Semen, in cervical mucus, 337  
storage, 329
- Semicircular canals, 259, 260, 261, 262
- Seminal vesicle, 316, 328, 329, 330, 329-331
- Seminiferous tubules, 317-321  
atrophy and fibrosis, 321  
cryptorchid, 322  
straight, 318  
types, 319
- Sensation, taste buds, 150, 152  
thirst, 150  
(see Pain)
- Sense organs, cutaneous, 379, 380  
ear, 259-263, 263-264  
eye, 250-259, 263-264  
Krause's end bulb, 379  
Meissner's corpuscle (tactile), 372, 379, 380  
muscle spindles, 309, 309, 310  
taste buds, 150, 152
- Serous alveoli, lingual, 151, 152  
salivary, 148, 148
- Serous cells, salivary, 148-149, 148
- Sertoli cells, 320, 321, 349
- Sex, hypothalamic center, 365  
determination by, tumors, 350  
chromosomes, 322  
hormones, 348  
hormones, 125, 324
- Sheath of Schwann, 236
- Shock, 66
- Shrinkage of tissue, artery wall, 60, 61  
dermis, 377, 378, 379-380  
follicular, 346  
large intestine, 171  
muscle nuclei, 304  
small intestine, 163  
spleen, 95
- Silver nitrate preparations, peritoneal surface, 165
- Sino-ventricular system, 80-81, 80
- Sinus node, 313
- Sinuses, lymphatic, 89-90, 89, 91, 92  
nasal, 202  
paranasal, 207  
venous, 97, 97, 99-100, 100, 102
- Sinusoids, hepatic, 190, 190, 192  
relation to capillaries, 73, 73
- Skeletal muscle, 305-312  
age changes, 310-312, 311  
androgenic hormone influence, 310  
atrophy, 310  
blood supply, 309  
compared with other types, 304  
contraction, 309, 309  
creatine content, 310  
cross-contractions, 306, 307, 308, 307, 309  
dark and light types, 308  
glycogen content, 310  
hypertrophy, 310  
lymphatic, 309  
motor end plates, 306, 309  
muscle spindles, 309, 310  
myofibrils, 308  
myosin, 308  
nerve endings, 309, 310  
paralyzed, 310  
sarcomere, 307  
water content, 312  
summary, 315
- Skin, 367-389  
adjustment, 387  
dermis, 377-383  
epidermis, 367-377  
functions, 387-389
- Skin, hairs, 382, 383-385, 388  
microincineration, 387  
nails, 386-387  
protection, 387  
sebaceous glands, 367, 376, 382, 383, 385  
sweat glands, 370, 385-386, 386  
summary, 387-389
- Small intestine, 162-170  
contracted and distended, 162, 163  
dissection of, 162  
duodenum, 165, 165, 170  
ileum, 167-168, 168, 169, 170  
jejunum, 167  
movements of villi, 162
- Smears, blood, 20  
vaginal, 357, 363, 364
- Smooth muscle, 303-305  
age changes, 305  
compared with other types, 304  
contraction bands, 173, 173  
hypertrophy, 305, 305  
tonus, 303
- Sneezing reflex, 207
- Sodium chloride, blood stream, 45  
in sweat, 386  
fluoride, effect on dentin, 141, 142
- Solutions, hypertonic, 42  
hypotonic, 42  
isotonic, 42
- Sound transmission, 262
- Spasm, traumatic arterial, 61
- Special (specific) endothelia, 103, 125, 129, 192  
192
- Specialization, basis of, 391
- Spermatids, 320, 321
- Spermatocytes, primary, 319, 320  
secondary, 319, 320
- Spermatogenesis, 319-324, 320, 323  
chromosomes, 321-322  
influence of temperature, 322-324  
waves of, 326
- Spermatogonia, 319, 320
- Sperms (spermatozoa), 321, 327  
discovery of, 337-338, 338  
ejaculation, 338  
fertilizing power, 337  
in cervical mucus, 337  
in Fallopian tube, 352  
motility, 337  
number, 338  
passage through tubules, 326  
phagocytosis of, 26-27  
production, 322  
storage, 329  
temperature susceptibility, 332  
variations in, 336, 337  
X and Y, 322
- Sphenoidal sinus, 207
- Sphincters, vascular in spleen, 99, 101, 101  
rectal, 173
- Spinal cord, reflex arc, 233, 233  
ganglia, sensory cells of, 231
- Spiral architecture in, arterial wall, 60, 61  
intestinal wall, 163  
perineurium, 236
- Spirochetes, gastric, 160
- Spleen, 95-103  
capsule, 95, 97  
compared with lymph nodes, 106  
with thymus, 106  
hemostatic, 101, 102  
lobules, 97, 97  
lymphatic tissue, 98, 99  
microscopic landmarks, 95, 96



- Sperms, Spermatogenesis)
- e, 125, 324, 348, 374, 376, 376
- rna, 344, 344
- trone), 324, 330, 330
- oduction, 363
- y glands, 360, 362
- 357, 359
- 24
- isation of, 150
- es, 104
- lin, 107
- 103-108
- ed with lymphatic tissues, 106
- 104, 104, 105
- al pearls, 106
- n, 107
- s corpuscles, 105, 106
- a, 104, 105, 105
- vidin, 107
- and old, 104-105, 104, 105
- try, 108
- 109-115
- of iodine, 109
- tion of, 114
- uorescence, 110
- l, 110
- ig of, 112, 113
- s, 110-111, 112, 113
- , 114
- thyroidism, 109, 112, 113
- thyroidism, 109, 112, 113
- thatics, 109, 110, 111
- vation *in vivo*, 114
- autographs, 112
- erature influence on, 115, 115
- otropic pituitary effect, 114, 114
- mary, 136
- in, 109
- et on fat, 280
- lines of arrested growth, 286
- e, definition of, 56
- e fluid, 13-16
- enal medulla, 127
- erior chamber eye, 253
- acket brigade," 349
- ehrosinial fluid, 246-247
- ifused with lymph, 11
- stors in diversity, 15
- mphocytes in, 31
- ruous system, 247
- utrophiles in, 25-26
- ntoneal, 154-155
- rmits specialization, 391
- al, 227
- olemic, 107
- ubarachnoid, 247-248
- olume adjustments, 391
- sue transparency, 254
- ngue, 150-153
- glands, 151, 152
- apillae, 151-152
- tiste buds, 150, 152
- nstils, 86-88, 87
- crypt, 88, 88
- lymphatic follicle, 87
- ncher, 202, 208, 208
- ran-formation, Graafian follicles to seminifer-
- ous tubules, 350
- lymphocyte to macrophage, 31, 32
- to monocyte, 31-35
- monocyte to macrophage, 36
- ransition band, adrenal cortex, 121
- ransitional epithelium, 231, 231
- ransparency, tissue, 254
- Transplantation, bone, 297
- endometrium, 364
- skin, 280
- (see Implantation)
- Trephones, 27
- Trialist concept of blood, 38
- Trichinosis, myocardium, 82
- Trypan blue vital stain, 102
- Trypanosome staining of, 102
- Trypsin, pancreatic, 184, 187
- Tubercle bacilli, phagocytized by monocytes, 34, 35
- Tuberculosis, lymphocytes in, 31
- Tumors, adrenal medullary, 127
- argentaffin, 167
- bone, 286
- endocrine, 136
- feminizing, 350
- glomus, 72
- interstitial cell, 325
- lipomas, 280
- lungs, 212
- masculinizing, 350
- melanomas, 375
- ovary, 350
- pancreatic islets, 185
- parathyroid, 119
- pineal, 135
- pituitary, chromophobe, 130
- skin, 372, 374
- testis, 350
- (see Cancer)
- Tunica, albuginea testis, 316, 317
- mucosa, gastric, 157
- serosa, gastric, 157
- vaginalis testis, 316, 317
- Turbinates, nasal, 203
- Twins, blood pressure, 71
- Tympanum, 259

## U

- ULTIMOBANCHIAL bodies, 114
- Ultraviolet rays on epidermis, 374
- Underfeeding, effect on, digestive tract, 170
- Paneth cells, 166
- thyroid and parathyroids, 120, 120
- Unitarian concept of blood, 38
- Upper alimentary tract, 137-153
- Urea, renal tubules, 228
- Ureters, 231
- Urethra, 231
- Urinary bladder, 231, 316
- Urinary system, 217-232
- nephrons, 219-226
- passages, 230-231
- renal lobes, 217
- lobules, 218
- ureters, 231
- urethra, 231
- urinary bladder, 231, 316
- summary, 232
- (see Kidney)
- Uterine artery, 62, 71
- tube, 340
- (see Fallopian tube)
- Uterus, 353-359
- endometrium, 353
- glands, 354, 355, 356
- lymphatics, 353
- menstrual cycle, 353-358, 355, 356, 361, 364
- muscular changes, 305
- myometrium, 353, 358
- spiral arterioles, 354





